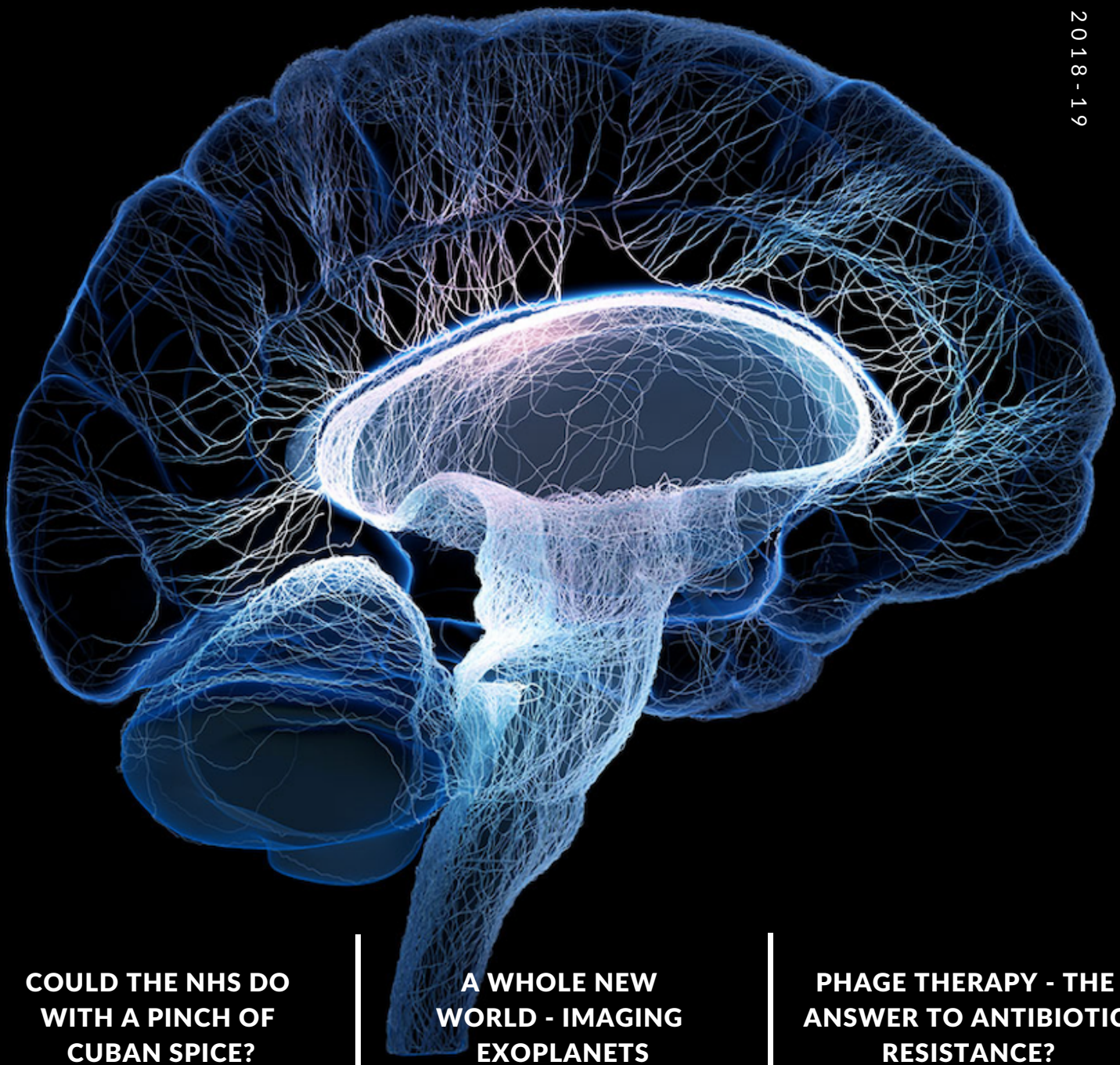
UgSJ

Undergraduate Science Journal
University of Exeter

VOLUME 3: ISSUE 1: 2018-19



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A Message from The Editors

We are delighted to share with you Volume 3, Issue 1 of the Undergraduate Science Journal. This edition features original, high-quality pieces of work from enthusiastic undergraduates from the College of Life and Environmental Sciences, the College of Engineering, Mathematics and Physical Sciences, and the College of Medicine and Health.

The objective of this journal is to publish interesting, up-to-date and original work carried out by University of Exeter undergraduate students in the fields of Science, Technology, Engineering, Mathematics and Medicine. We aim to improve the accessibility of undergraduate research, facilitate its promotion to the wider student community and ultimately to contribute to a greater interdisciplinary outlook across scientific fields at the University. As this issue and previous issues demonstrate, the work produced by students is innovative, thought-provoking and wide-ranging in speciality – and we hope that the journal brings such work to a greater limelight! The journal is printed annually and distributed across all campuses at the University of Exeter – Streatham, St Luke's, Penryn and Truro.

We have received an overwhelming number of submissions to the journal this year and we are enormously grateful for every submission we have received. Moreover, we are indebted to all the authors, peer reviewers and staff whose contributions have made the publication of this issue possible.

As the current committee, we have thoroughly enjoyed co-ordinating the running of the journal during this academic year. We hope you enjoy reading this latest issue.

The Editors.

Abbie Macmillan-Jones
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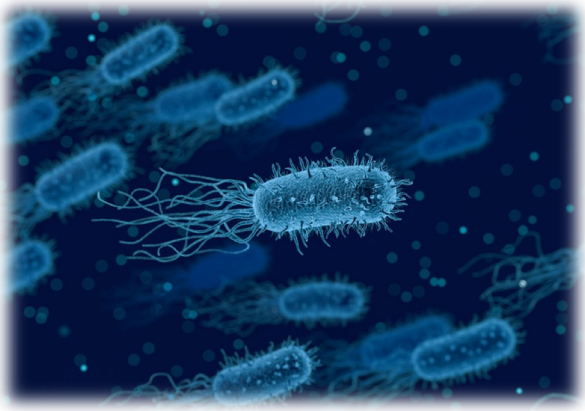

MICROBIOLOGY

CEMPS

VOLUME 3 (2018-2019)

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Pharmacological Management of Lewy Body Dementia

Anastasia Oates

Introduction

Dementia with Lewy Bodies (DLB) and Parkinson's disease dementia (PDD) are neurodegenerative diseases caused by build-up of Lewy bodies in the brain. Lewy bodies (named after German Neurologist Freiderich H. Lewy in the early 1900's) are abnormal deposits of alpha-synuclein protein, which accumulate and interfere with the transmission of neurotransmitters including dopamine and acetylcholine (Neef 2006). Although separate, these diseases share similar pathophysiology and features, so are therefore grouped under the umbrella term 'Lewy Body Dementia' (LBD).

LBD constitutes the second most common cause of dementia after Alzheimer's disease (McKieth, 2004). It typically manifests as: fluctuating cognitive impairment, Parkinsonism (slow movement, rigidity and tremor) and visual hallucinations. Impairment of executive function and visual perception, autonomic dysfunction (hypotension, loss of consciousness) and REM sleep disorders are other classic features (Dizon 2018).

The neurodegenerative, motor and autonomic symptoms significantly impair quality of life for the patient, often leading to debilitation, isolation and depression. It is known that imbalance and dysregulated transmission of neurotransmitters dopamine, acetylcholine and glutamate in the central nervous system (CNS) are responsible for the movement disorder and dementia in LBD, yet pharmacological management of LBD is notoriously difficult and treatment success is low. Anticholinergic drugs (which block the effect of acetylcholine on motor neurones to prevent involuntary muscle movements) and dopamine agonists (which mimic the effect of dopamine in order to regulate movement and reduce psychological disturbance) used to control the physical symptoms of Parkinsonism commonly cause psychosis in patients with LBD. However, antipsychotics used to treat other types of dementia can cause fatal adverse reactions in patients with LBD (Neef, 2006). Recently, Donepezil and Rivastigmine, (acetylcholinesterase inhibitors (AChEI) which inhibit the enzyme that breaks down acetylcholine) have shown benefit in patients with LBD with substantially fewer side effects. In addition, Memantine (which blocks the action of neurotransmitter glutamate (known to exacerbate dementia)) has also yielded positive results. Using the available evidence, this essay aims to review the literature surrounding the pharmacological management of Lewy body dementia.

Method

In order to optimise relevance, accuracy and reliability, only studies from trusted scientific journals, with at least 50 patients and published within the last 20 years will be included. Recognized scoring systems which provide quantifiable and comparable data are preferred, such as; Mini Mental State Exam (MMSE) which tests working and episodic memory, Neuropsychiatric Inventory (NPI) which assesses psychiatric disturbance and agitation, Alzheimer's Disease Assessment Scale–cognitive subscale (ADAS-cog) which assesses cognition and Alzheimer's Disease Cooperative Study Clinical Global Impression of Change (ADCS-CGIC) which measures observer's impression of improvement in cognitive, functional and behavioural performance. When comparing data, only data with a confidence interval >95% and p value <0.05 will be included. All studies must be ethically-approved and subjects must fulfil clinically diagnostic criteria for LBD.

Donepezil

Donepezil is an AChEI which prevents acetylcholine from being broken down in the CNS, meaning that levels are less likely to become depleted and cause symptoms. Three randomized placebo-controlled studies (Mori et al. 2012, Ikeda et al. 2015 and Dubois et al. 2012) showed positive results in patients with LBD. Patients taking 5mg or 10mg Donepezil demonstrated a significant improvement in MMSE score compared to the placebo group (5mg: mean difference, 1.5-3.8; 10 mg: mean difference, 1.72-2.4). Improvements were also seen in ADAS-cog scores in the 5mg and 10mg groups (-2.45 and -3.72 respectively) compared to placebo (-0.3). In addition, improvements in assessment of verbal fluency and attention in groups treated with 5mg or 10mg of Donepezil were identified (Dubois et al. 2012). However, no study found significant improvement in NPI or Disability Assessment of Dementia (DAD) score (which measures functional ability) - indicating that the psychiatric and debilitating physical symptoms of the disease were not lessened. Treatment regimens varied from 12-24 weeks, with subjects returning to baseline scores on withdrawal of the drug.

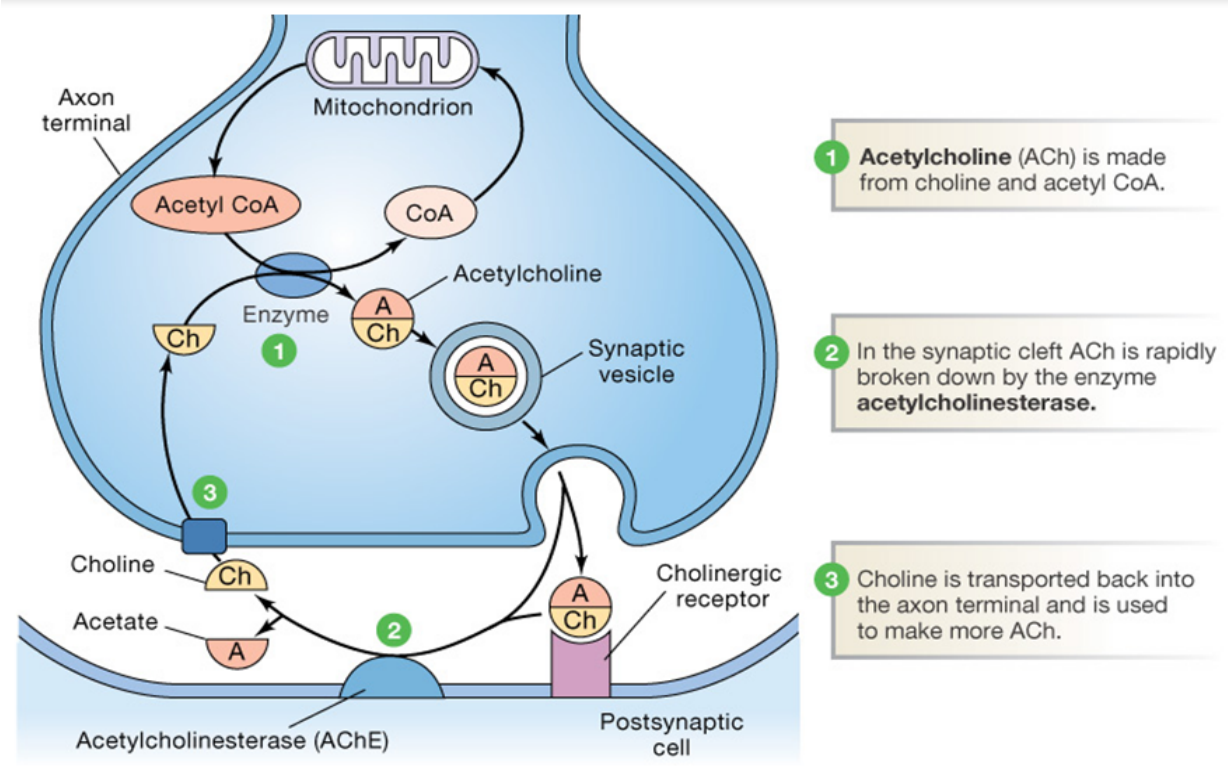

Figure 1. *Peaknootropics.com, Acetylcholinesterase an Memory Problems, available online at: https://peaknootropics.com/acetylcholinesterase-memory-problems/?v=79cba1185463*

Adverse reactions to Donepezil were noted across all studies and included nausea, vomiting and diarrhoea. There was a higher rate of discontinuation in Donepezil-treated patients compared to placebo (Dubois et al. 2012). Slight worsening of dyskinesia was noted across active and placebo treatment groups in two studies, which likely reflects natural progression of the disease. The incidence of Parkinsonism was higher in the 10mg Donepezil group (Ikeda et al, 2015), which is potentially concerning and warrants further investigation. Overall, donepezil was well tolerated in patients with DLB and has shown to improve memory and cognition. With careful attention to gastrointestinal upset and worsening of movement disorder, patients can safely benefit from Donepezil (Ikeda et al, 2015).

Rivastigmine

Two large, randomized placebo-controlled trials (Emre et al. 2009, McKieth et al. 2000, and Wesnes et al. 2002) tested the efficacy of Rivastigmine (which is also an AChEI and has the same mechanism of action as Donepezil) in LBD. Patients were given 3-12mg of Rivastigmine daily for 20-24 weeks. Patients taking 3-12mg of Rivastigmine daily had a mean improvement of 2.1 points in score for the 70-point ADAS-cog, from a baseline score of 23.8, as compared with a 0.7-point worsening in the placebo group, from a baseline score of 24.3 (Emre et al 2009). An improvement in NPI score was seen across both studies; 45.4-63.0% of Rivastigmine vs. 30.0-34.6% of placebo patients showed at least a 30% improvement from baseline NPI score (McKieth et al. 2000, Emre et al. 2004). Improvements were seen in levels of apathy, anxiety, delusions, hallucinations, and agitation. No significant differences in MMSE score were found in any study. However, there was significant improvement on CDR (computerised cognition assessment) reflecting an improvement in attention, working memory and episodic memory. Patients given placebo showed a significant deterioration of 19% at 20 weeks, whereas patients on rivastigmine improved by an average of 23% (Wesnes et al. 2002). The predominant adverse events were nausea, vomiting, anorexia and somnolence. These were significantly higher in the Rivastigmine group than in those on placebo. Worsening of tremor was also seen in Rivastigmine patients in one study (Emre et al. 2004). Three weeks after discontinuation, most parameters of cognitive performance returned to baseline and side-effects disappeared. This shows that Rivastigmine was generally well-tolerated and could improve neuropsychiatric symptoms, but not cognition and memory loss.

(Emre et al 2010 and Aarsland et al 2009) have revealed some benefit in patients with LBD. In both studies, patients were randomly allocated to 20mg Memantine daily or placebo daily for 24 weeks.

At week 24, patients with DLB who received Memantine showed greater improvement according to ADCS-CGIC (Clinical Global Impression of Change) scores than those who received placebo (mean change from baseline 3·3 VS 3·9, respectively) (Emre 2010). This was also seen (to a lesser extent) in the Aarsland et al. 2009 study; patients in the Memantine group had better CGIC scores after 24 weeks than those taking placebo (mean difference = 0·7). Although statistically significant, it is arguable that these differences in score are negligible, especially in a smaller sample size.

NPI scores showed significantly greater improvement in the Memantine group than in the placebo group (−4·3 VS 1·7) in patients with DLB, but not in those with PDD (−1·6 VS −0·1). This evidence suggests that Memantine could be beneficial for patients with DLB, but not PDD. Despite the fact that improved speed on attention-based tasks in the Memantine group was found in one study (Aarsland et al. 2009), there were no significant differences between the groups in secondary outcome measures: MMSE, DAD, Unified Parkinson's Disease Scale or Zarit Caregiver Burden Scale (which assesses the strain placed on the caregiver).

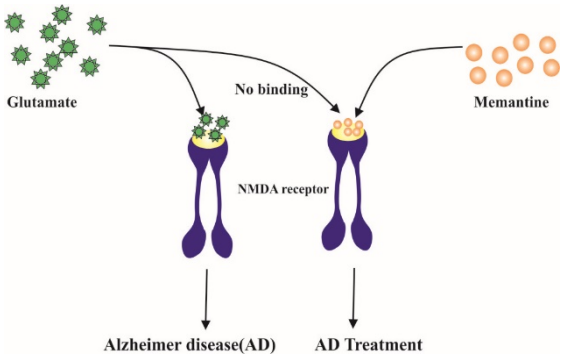

Figure 2. Goyal M., 2018, Memantine, Drugs Details, available online at: https://drugsdetails.com/memantine/ [accessed 10/03/2019]

Although Memantine has shown some benefit for Alzheimer's disease, it seems to produce no significant improvement for patients with LBD. Nevertheless, adverse events were minor and similar between the placebo and treatment groups.

Discussion

There was a lack of trials which fitted the criteria of this review. Although these were all randomized placebo-controlled trials, only one was double-blinded and most had less than 200 subjects. This evidence was published in reliable, non-biased medical journals, although some featured the same authors and two papers referred to the same trial (McKeith et al. 2000 and Wesnes et al. 2002). Some trials were only 12 weeks long, which is arguably not long enough to properly assess the benefit or harm of a drug. Moreover, results may have been influenced by subjects' pre-existing medications and medical conditions.

Performance in pre and post testing exercises could easily be affected by the natural fluctuation of cognition and attention, which is a hallmark of DLB. This could also explain why in some studies the placebo groups improved or deteriorated. Increased caregiver education and social interaction for the patient could also influence results. Both these reasons call for longer treatment periods with more frequent assessments.

Overall, it would appear that Donepezil and Rivastigmine offer the most potential for improving symptoms of LBD. 5-10mg daily of Donepezil can improve MMSE score, but not the psychiatric disturbance or behavioural problems. 3-12mg of Rivastigmine daily can improve behaviour and psychiatric symptoms, but shows little evidence for improving cognitive function. It would be interesting to trial combination therapy. Both drugs have shown similar side-effect profiles which were relatively mild and tolerated well by most patients. Despite being well-tolerated, Memantine showed no real benefit for patients with LBD.

Recently, other drugs Tacrine (Querfurth et al 2000) and Galantamine (Litvinenko et al 2008) have shown to improve NPI scores, MMSE scores and psychosomatic symptoms. There is a need for larger and longer trials of other types and combinations of drugs, with consistent and sensitive measurement tools of cognitive function, impact on activities of daily living and psychological well-being. This would help to produce more robust evidence of medications that could improve the lives of sufferers of LBD.

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An evaluation of sodium-potassium ATPase ion pump dysfunction in Alzheimer's disease

Scarlett Parr-Reid

Abstract

Sodium-potassium ATPase pumps (which use ATP as an energy source) maintain a constant charge inside cells, by regulating of the numbers of sodium ions leaving and potassium ions entering. The consequent ion ratio is known as the resting membrane potential. This review evaluates evidence for sodium-potassium ATPase dysfunction in Alzheimer's disease (AD). Recent research on mouse brain cells (models of human brains) and those from Alzheimer's patient's post-mortem shows that the protein ß-Amyloid (Aß) lodges between the pump's sub-units, blocking their interaction. Resultantly, membranous pores form (cheese-like holes) allowing excessive potassium and sodium ions into cells. This review discusses the cerebellum, frontal cortex, parietal cortex and cerebrospinal fluid of the brain to understand the effects of Aß with respect to location, concentration and sodium-potassium ATPase inactivation. Understanding the mechanism behind AD facilitates more accurate diagnoses and testing for effectiveness of potential drug treatments. To achieve this, larger scale clinical trials on Alzheimer's-affected patients are necessary. Although research is in its infancy, knowledge of the interaction between Aß and sodium-potassium ATPase in the future may enable early intervention.

Introduction

50% of the brain's energy expenditure is devoted to Sodium-potassium pump activity, making it an essential transmembrane enzyme in human cells (Rose and Valdes, 1994). It transports ions using energy released from Adenosine Triphosphate (ATP) molecules when they're split by water. This creates an altered charge difference between the inside and outside of cells, known as a potential difference, allowing nerve signal conduction in response to stimuli, and ion and nutrient transport across membranes. The resting membrane potential (charge of -70 millivolts) is an ion-dependent voltage difference across the cell membrane achieved via ion concentration gradients, for optimal cell function. In healthy cells, sodium-potassium ATPases use ATP energy to transport 3 sodium ions out of the cell and 2 potassium ions in (De Lores Arnaiz and López Ordieres, 2014).

Alzeihmer's Disease (AD) is neurodegenerative and is the most common cause of Dementia. It causes gradual dysfunction of memory and cognition (Zhang et al., 2012). The prevalence of the disease is growing, expected to reach 75.6 million people by 2030, increasing the necessity for treatment (World Health Organisation, 2012).

AD involves Aß peptide plaque formation from the mutated Amyloid Precursor Protein (APP). APP normally concentrates at synapses (junctions between neurons) as a regulator of synaptic formation (Ntsapi et al., 2018). APP is abnormally cut by α, ß and γ secretase enzymes to form α, ß-40 and 42 peptides, the latter of which builds up to form Aß protein aggregates. Insertion of mutated Aß between sodium-potassium ATPase sub-units forms pores, causing the resting membrane potential to be too positive, due to leakage of these positive ions. This prevents signaling at cell junctions normally reliant upon negative charge (inhibition), the focus of this review. Aß exists in different forms, distinguished by their lengths (for example 1-40 and 1-42 amino acids long). Not all forms impact upon sodium and potassium ion balance, due to their length differences and their conformation between the pump sub-units.

To investigate sodium-potassium ATPase function in AD, scientists have studied mouse brain cells to observe levels of the sodium-potassium ATPase activator, glutamate, as well as the pump's α, ß and γ sub-unit functions in the frontal cortex, parietal cortex, cerebellum and cerebrospinal fluid (Vitvitsky et al., 2012). Studying the relationship between increased Aß concentration and sodium-potassium ATPase activity has revealed a gradual pump dysfunction. This effect is compounded, since there is a myriad of alternative protein forms of sodium-potassium ATPase in the brain, there is an increased chance of Aß pathological impact in AD. However, these findings merit larger clinical trials to enhance validity and accuracy.

The sodium-potassium ATPase complex contains 2 of each of the α, ß and γ sub-units. The catalytic α subunit contains sodium and potassium binding sites, positioned and stabilised by the ß subunit (Yan and Shapiro, 2016. The γ subunit regulates sodium-potassium ATPase pump activity in a tissue-specific manner, though its specific role is to be further explored. For example, the γ subunit can stimulate channel activity when expressed without α/β subunits, implying that its role may be independent from those needed to maintain normal sodium-potassium ATPase activity (Minor et al., 1998).

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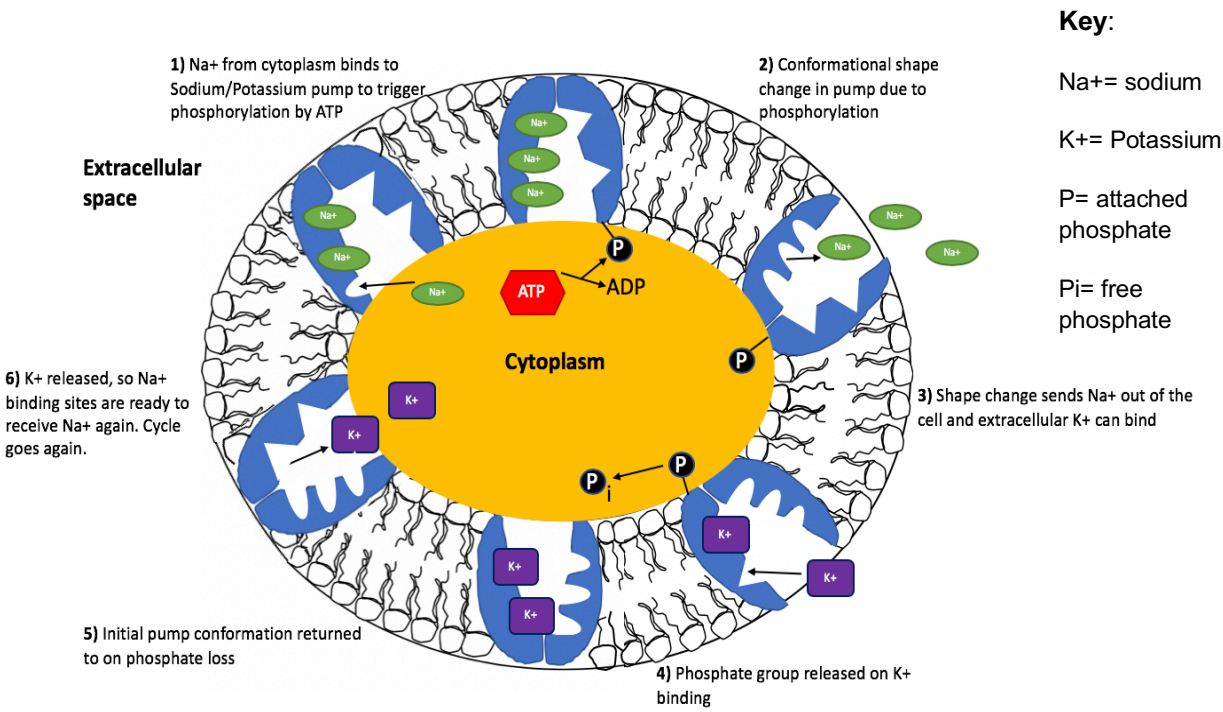


Figure 1. Sodium-potassium pump in action. Adapted from Zueva, T. 2017.

The pump enters different conformational states whilst active; increased binding affinity for sodium causes the sodium and potassium sites to face the cytoplasm, and increased binding affinity for potassium subsequently leads to these sites facing the extracellular region, as seen in Figure 1 above. 3 protein forms of the pump exist in mammalian brains: α 1, 2 and 3, increasing opportunity for pathology (SJ and Brady, 1999).

Astrocytes are glial cells - specialised in the brain and spinal cord to induce and maintain the blood brain barrier (the border between brain tissue and the peripheral blood) (Ballabh, Braun and nedergaard, 2004). They signal to nearby specialised cells called endothelial cells and pericytes of the central nervous system blood vessels (Herndon, Tome and Davis, 2017). The presence of Aß can disrupt transmission by astrocytes, uptake of neurotransmitter signal molecules and calcium ion signalling in astrocytes (González-Reyes et al., 2017). Brain neurons rely on astrocyte activity, so are easily affected by altered astrocytic function.

Aß plaque formation is due to abnormal APP breakdown, during Aß formation. Research shows that increased sodium and potassium concentrations in AD are not due to increased excitation (that is the improper enhancement of a response), but impaired synaptic inhibition (that is an improperly decreased response), disrupting learning and memory (Perez, Ziburkus and Ullah, 2016).

Discussion

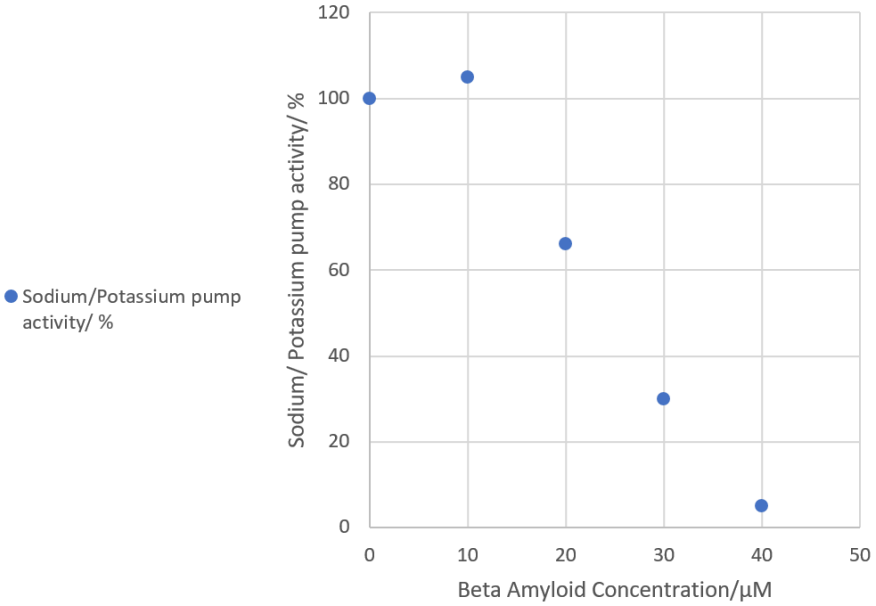
The glutamate-glutamine cycle of cells activates sodium-potassium ATPase, during which one potassium ion leaves and 3 sodium ions enter for every glutamate amino acid (main excitatory neurotransmitter in the brain) entering (Vitvitsky et al., 2012). Studies on mice with AD show that Aß exposure decreases glutamate uptake compared to controls, increasing the concentration of available glutamate in the synaptic cleft between neurons. This can result in overexcitability, as glutamate promotes activation in learning and memory signalling. However, the results are inconsistent in Vitvitsky et al's paper. Despite slightly raised glutamate levels in the frontal cortex, parietal cortex, cerebellum and cerebrospinal fluid compared to healthy controls, evidence from Vitvitsky et al is statistically insignificant. For example, AD brains from patients post-mortem present outer cerebellum sodium imbalances and potassium imbalances, yet no cerebrospinal fluid imbalances, indicating that Aß has specifically intracellular effects. Nevertheless, AD patients show twice the healthy intracellular sodium concentration and 8-15% higher potassium concentration inside cells post-mortem (Vitvitsky et al., 2012).

Astrocytes - the most abundant human brain cell – show altered activity in the presence of Aß (as mentioned in the introduction above), helping us to observe effects of Aß treatment in mice (Perez, Ziburkus and Ullah, 2016) (Herndon, Tome and Davis, 2017). This assertion is consistent with trends presented by astrocytes from excised

brains of 12-16 month old mice grown with Aß in Perez, Zikurkus and Ullah's study; they show a 2-3x intracellular sodium concentration increase and a 1.5x intracellular potassium concentration increase (Perez, Zikurkus and Ullah, 2016).

However, disparity between human and mouse potassium concentration increases merits further investigation into the maximum actual potassium concentration increase that occurs in AD. The efficacy of Perez, Zikurkus and Ullah's study is enhanced by observing the Sodium/Potassium concentrations in astrocytes treated with increasing Aß concentrations up to a maximal 50 micromolar (µM) with identical concentrations in culture medium. Western blot protein detection analysis of results showed decreased sodium-potassium ATPase expression in mildly or repeatedly Aß-treated astrocytes – indicative of a concentration-response relationship, respectively. Although, correlation doesn't always imply causation, as applying a sodium-potassium ATPase pump inhibitor (to decrease pump activity) only increases the intracellular sodium concentration, whilst decreasing intracellular potassium concentration (Vitvitsky et al., 2012). As such, sodium-potassium ATPase inhibition alone cannot account for both increased sodium and potassium concentrations in Aß-treated astrocytes, so scepticism remains around the true cause of AD pump inhibition.

Further work by the same group reveals Aß specificity, solely affecting brain astrocytes. While an astrocytoma cell line demonstrated a similar response to Aß as astrocytes, human embryonic kidney epithelial cell lines don't (Vitvitsky et al., 2012). Furthermore, longer Aß sequences (1-42 amino acids) don't alter sodium-potassium ion balances, yet shorter (1-40 amino acid sequences) do. As such, both Aß composition and location determine AD pathology. Currently unknown, the mechanism of Aß aggregation in sodium-potassium ATPase pathology was investigated experimentally (Petrushanko et al., 2016). Aß (1-42 amino acids long) binds between α and ß sub-units of the $\alpha 1 \beta 1$ sodium-potassium ATPase form. This creates a complex, blocking their interaction. Aß then forms hydrogen bonds and ionic bonds attaching to the sub-units, inhibiting sodium-potassium ATPase enzyme activity. This limits ion transport, which is beneficial in regulating short-term sodium-potassium ATPase activity (as it prevents a highly negative voltage across the membrane - hyperpolarisation) but causes long-term hyperactivity and neuronal dysfunction (Vitvitsky et al., 2012).
Aß concentration varies between cells, as in cultures with below sub-µM Aß concentration, cell specialisation is impacted. Whereas, at µM concentration, Aß is toxic (Petrushanko et al., 2016). Research by Petrushanko et al. depicts that Amilospheroids (tiny spherical Aß monomers) inhibit sodium-potassium ATPase hydrolytic activity only in



latter stages of AD; absent during early stages. In fact, these results show that 40µM Aß nearly completely inhibits sodium-potassium ATPase observed in model purified duck salt glands (organs containing excess salts). It's estimated that 24µM Aß elicits this. Interestingly, dilution of Aß concentration from 40µM to 10µM recovers some sodium-potassium ATPase activity. Intracellular potassium concentration influx becomes suppressed by 65% compared to controls.

Increasing Aß concentration decreases sodium-potassium ATPase activity, as shown in figure 2 above. Evidence from Aß treated mice supports this correlation, where a mutated APP gene called APdE9 is expressed (Perez, Ziburkus and Ullah, 2016). As sodium and potassium enter cells, the ionic charge difference between the outside and inside, established at resting membrane potential, decreases. Resultantly, there is a more positive voltage inside the cell, which reduces synaptic inhibition - an important consideration for managing excitotoxicity of AD. This indicates usefulness of Aß pore blockers such as the NA7 peptide and Bexarotene. (Arispe, Diaz and Simakova, 2007) (Babak, 2015). NA7 peptide blocks sodium and potassium entry by mimicking part of the Aß pore that facilitates entry, as such decreasing ion leakage to 0 in 3 minutes. Bexarotene increases APO-E gene expression (needed for Aß

breakdown). However, bexarotene has demonstrated little effectiveness, and with undesirable effects of high blood cholesterol and triglyceride levels, decreased thyroid activity and a lack of the primary effect in improving cognition following 3 months' administration associated with its use, it's not recommended to patients. Although, NA7 peptides are promising, achieving full protection from Aß toxicity to astrocytes at low doses with no specific side effects (Babak, 2015). Regardless, research into alternative molecular mechanisms of sodium-potassium ATPase pathology and more extensive clinical trials will crucially determine dosage, side effects and interactions in broader populations of AD patients (Hoyt, 2017). Though, the feasibility of clinical trials carried out is limited by high costs, potential side effects and lack of willing participants.

Conclusion

There is sufficient evidence supporting the fact that Aß plaque aggregation, to form sodium and potassium-permeable pores in the plasma membrane, lowers synaptic inhibition, resulting in cognitive impairment during AD. With pathology present in mouse models at Aß concentrations as low as 24µM, treatment must focus on intervention within the early stages of Alzheimer's neurodegeneration (Petrushanko et al., 2016)

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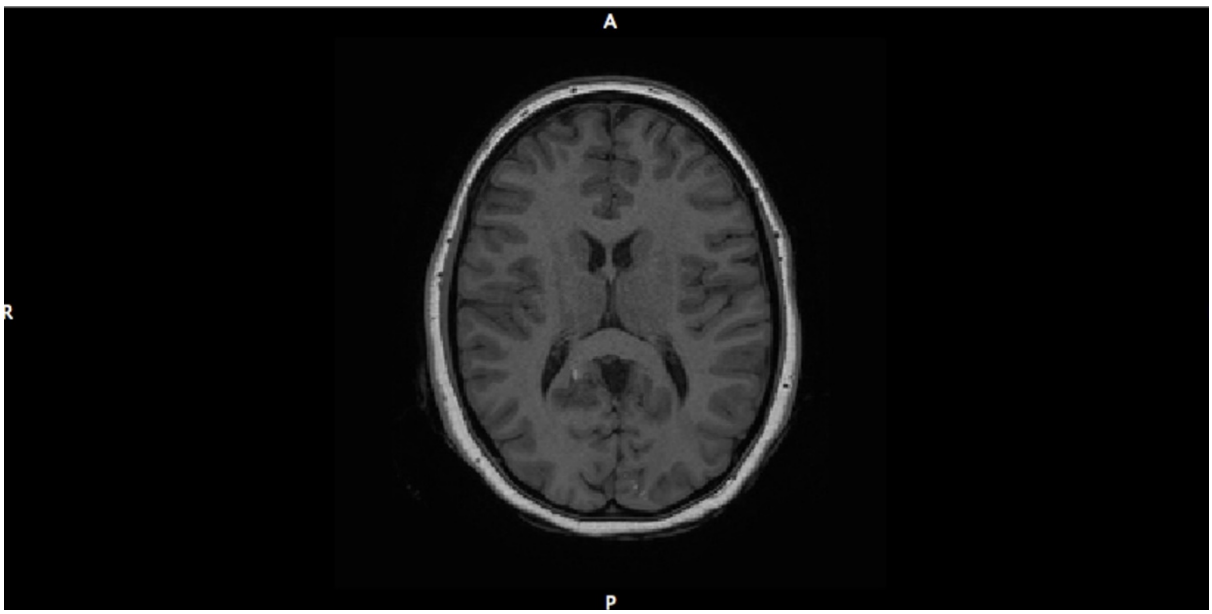
Scientific Art and Photography:

Photo of a male mallard duck *(Anas platyrhynchos)* captured mid-flight.

Photo of a herring gull *(Larus argentatus)* captured mid-flight at Lulworth cove.

Solar eclipse over the Marshall Islands in July 2009 photographed by Miloslav Druckmuller.



T1-weighted MRI Scan of a human brain (20 year old female).

Fallow deer in North Yorkshire, autumn 2016.

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The Molecular Mechanisms behind ß-lactam antibiotic resistance in Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Aris Alexiadis

Abstract

Antibiotic resistance poses a huge threat to society. The inappropriate use of antibiotics has resulted in the development of bacterial species such as Methicillin-resistant staphylococcus aureus (MRSA) which are resistant to β-lactams, one of the main antibiotics. These antibiotics function by inhibiting peptidoglycan cross-linking, which is essential for bacterial cell wall synthesis. Mechanisms of MRSA resistance to β-lactams include modification of the β-lactam target transpeptidase to prevent β-lactam binding, production of β-lactamase enzymes to induce degradation of β-lactams prior to binding to their target site, and production of cell wall techoic acid molecules which enhance resistance through controlling localization of proteins which promote β-lactam resistance. These mechanisms make anti-MRSA drug development challenging and highlight the need for synergistic therapeutics. This review explores how MRSA withstands β-lactam treatment on a molecular level, and how this can lead to identification of new drug targets and the restoration of β-lactam effectiveness against MRSA.

Introduction

Threat of Antibiotic Resistance

Antibiotic resistance poses one of the greatest global challenges to healthcare providers, with conventional therapies being rendered useless by certain bacteria (Watkins & Bonomo, 2016). In a report commissioned by former UK Prime Minister David Cameron in 2014, it was found that, without new and effective therapies, antibiotic resistance would be responsible for 10 million deaths worldwide per year by 2050 (O′ Neill, Jim, 2016). The development of antibiotic resistance is attributable to various factors, ranging from improper prescription in daily human medical practice to their overuse in the agricultural industry (Watkins & Bonomo, 2016).

One of the most common pathogenic bacteria is *Staphylococcus aureus* (Lowy, 2003). Penicillin, the first β-lactam antibiotic, was discovered in the 1940s, and subsequently used to treat *S. aureus* infections (Lowy, 2003). Throughout the 1950s Penicillin resistance developed in certain strains such as *S. aureus* phage type 80/81 (Gillespie & Alder, 1957). To combat this, methicillin, a derivative of penicillin was developed. Further resistance developed, and in 1961 the first strain of Methicillin-resistant *S. aureus* (MRSA) was isolated (Jevons, 1961). As MRSA infections are often contracted in hospitals, the increase in morbidity and mortality amongst patients raises global problems in healthcare (Baines *et al.*, 2015). MRSA prevention and treatment has higher costs than standard infection treatment due to the requirement for patient quarantine and specialist equipment (Athanasakis *et al.*, 2014). To mitigate the contraction risks and cost it is necessary to develop more efficient strategies to prevent antibiotic resistance.

Action of β-lactams and MRSA resistance

Methicillin and other β-lactam antibiotics target bacteria through inhibiting transpeptidase activity, which is responsible for the final step of cell wall synthesis. Inhibition occurs through binding of β-lactams to transpeptidase Penicillin Binding Domains (PBDs) on Penicillin binding proteins (PBPs) (Page, 1984). Defective cell wall formation leads to bacterial cell death (Bush & Bradford, 2016). However, molecular mechanisms have evolved which render MRSA resistant to β-lactams (Kaur & Chate, 2015). This has culminated in 95% of MRSA cases worldwide being unresponsive to first round antibiotic treatment (Kaur & Chate, 2015). This review considers the various molecular mechanisms which allow MRSA to resist β-lactam antibiotic therapy. It investigates the importance of the mecA gene producing the resistant PBP2a protein, the β-lactam-degrading β-lactamase enzyme and discuss emerging relevance of wall techoic acids (WTAs).

Discussion

MecA

MRSA resistance to β-lactams is thought to derive from the inheritance of a mobile genetic element called the staphylococcal cassette chromosome mec element (SCCmec) from another species, *Staphylococcus sciuri*. The SCCmec cassette facilitated expression of the mecA gene, producing an altered penicillin binding protein, PBP2A, as shown in Figure 1 (Rolo *et al.*, 2017). Unlike other PBPs, which are normally inhibited by β-lactams, PBP2a has a low affinity for β-lactams and is therefore able to facilitate peptidoglycan crosslinking in the presence of β-lactams and compensates for the inhibition of the β-lactam-sensitive PBPs (Rolo *et al.*, 2017). PBP2a, unlike other PBPs, has an additional molecular feature, which permits the entry of specific molecular structures to the PBP

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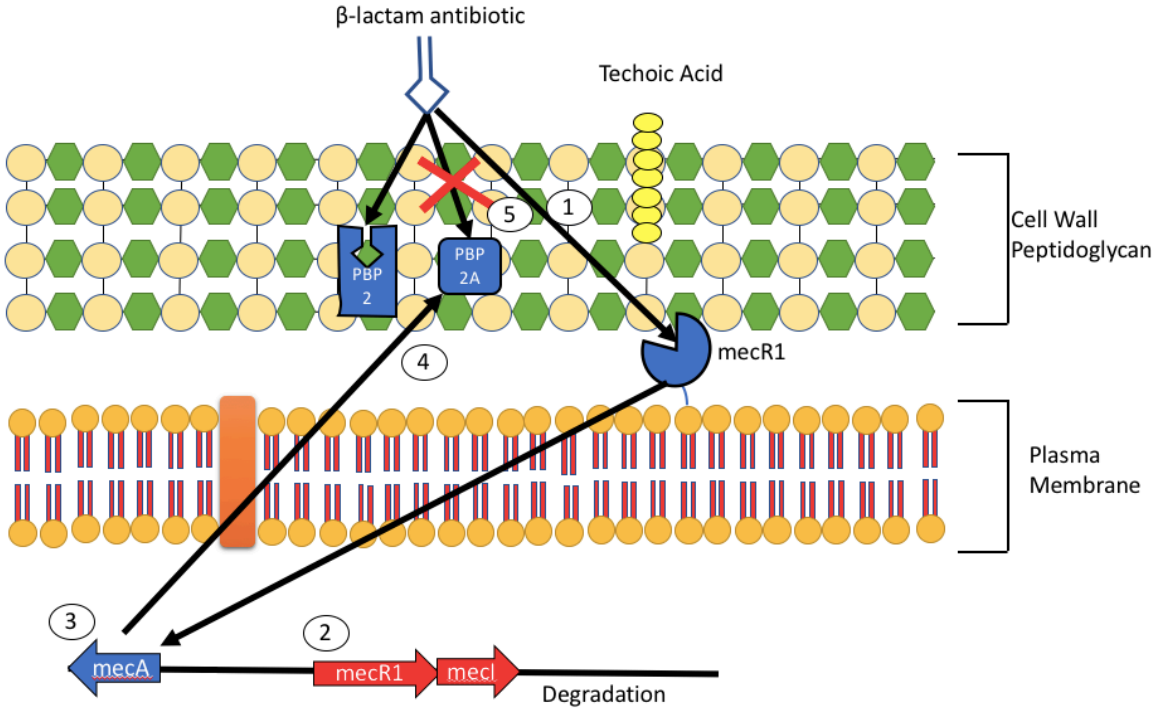

Figure 1. Schematic diagram representing PBP2a activation by the mecA gene. The β-lactam binds to the mecR1(1), leading to the degradation of mecA repressors (mecR1 and mecI) (2) facilitating mecA activation (3). PBP2a is then produced (4), leading to β-lactam ineffectiveness due to its low-affinity (5) (Hao *et al.*, 2012).

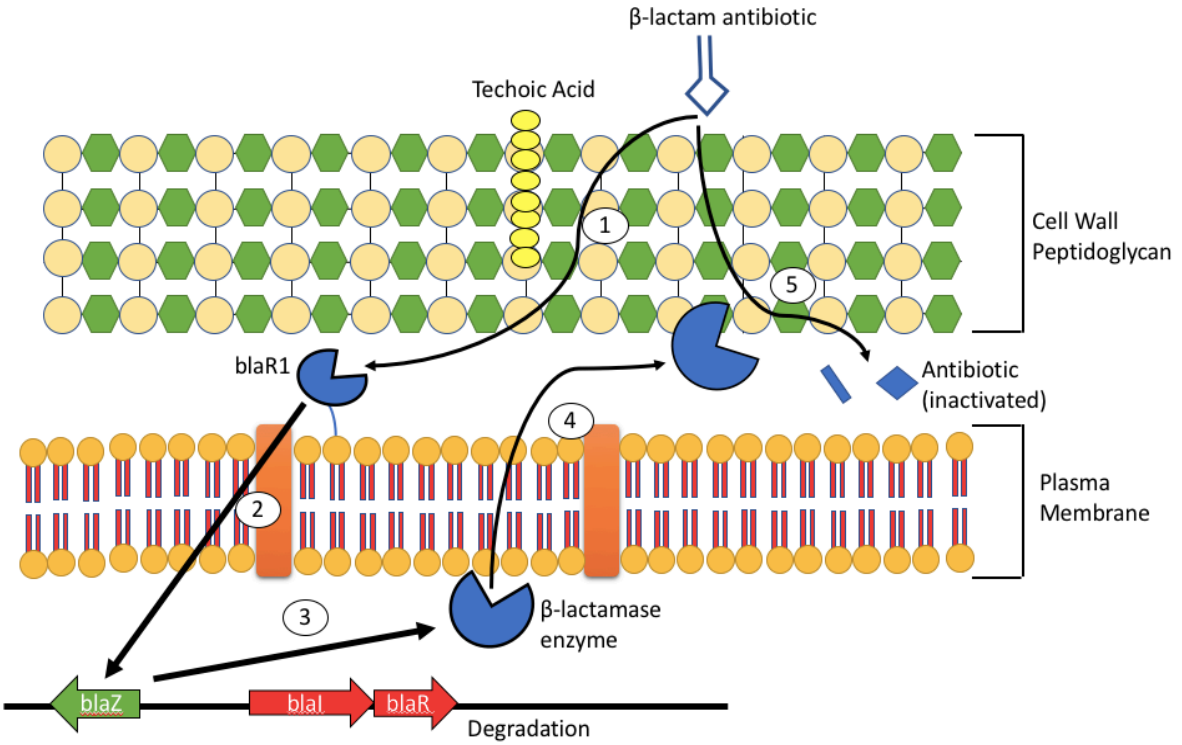

Figure 2. Schematic diagram representing WTA synthesis in Methicillin-Resistant *Staphylococcus Aureus*. Production is initiated by the transporter, TarO (1) and further facilitated by Tar enzymes (A, B, F, L, S and M) (2). The techoic acid is then transported to the peptidoglycan by TarGH (3) (Pasquina,Santa Maria & Walker, 2013).

active site (Mahasenan *et al.*, 2017). β-lactams however, cannot bind to this site, and it is only cell wall molecules muramic acid and peptidoglycan that can bind (Mahasenan *et al.*, 2017). Other β- lactam-based inhibitors such as ceftaroline have been developed which are able to bind to the site to facilitate PBP2A active site opening, thereby permitting normal β-lactam binding and action (Srisuknimit *et al.*, 2017).

While the identification of mecA is still the principal method of identifying MRSA infection, there have been reports of human MRSA isolates that which have no detectable mecA, but are still resistant to β-lactam treatment (Ba *et al.*, 2014). This suggests that alternative mechanisms for resistance in MRSA exist. However, the number of isolates used in this study was insufficient to provide substantial evidence for this (Ba *et al.*, 2014). Additionally, the gene study excluded other genes that are thought to confer antibiotic resistance to MRSA (Ba *et al.*, 2014). In addition, mecC, a similar gene although currently quite rare, presents a problem for MRSA diagnosis as mecC MRSA bacteria are still resistant to β-lactams without needing to have mecA incorporated into the bacterial DNA (Paterson, Harrison & Holmes, 2014). This suggests that the treatment of different varieties of MRSA is more complex than those for which current treatments allow.

β-lactamase

In the presence of β-lactams, MRSA may produce the enzyme β-lactamase. Detailed in Figure 2, β-lactamase is situated in the space between the bacterial plasma membrane and the peptidoglycan cell wall. This enzyme cleaves the β-lactam molecule, disabling the drug's ability to bind to the PBPs present in the peptidoglycan (Peacock & Paterson, 2015).

The β-lactamase enzyme is produced by the blaZ gene and is regulated by the blaR-blaI-blaZ system (Blázquez *et al.*, 2014). The repressors and inducers of β-lactamase in this system are very similar to the genes which regulate mecA. In addition, messaging between mecA and bla-related genes may occur (Blázquez *et al.*, 2014). Presence of the bla-containing DNA stabilizes and enhances mecA action, thereby further highlighting the collaborative nature between the blaZ and mecA genes and increasing the likelihood of MRSA resistance to β-lactams (Cohen & Sweeney, 1973). However, the exact molecular mechanism behind the cross-talk pathway has yet to be characterised and confirmed, as not all reports agree that the presence of a blaZ gene enhances mecA expression (McKinney *et al.*, 2001). It has also been suggested that while the blaZ gene inhibits mecA expression in conjunction with mecR1 and mecI, it does not activate it in conjunction with mecR1 (McKinney *et al.*, 2001).

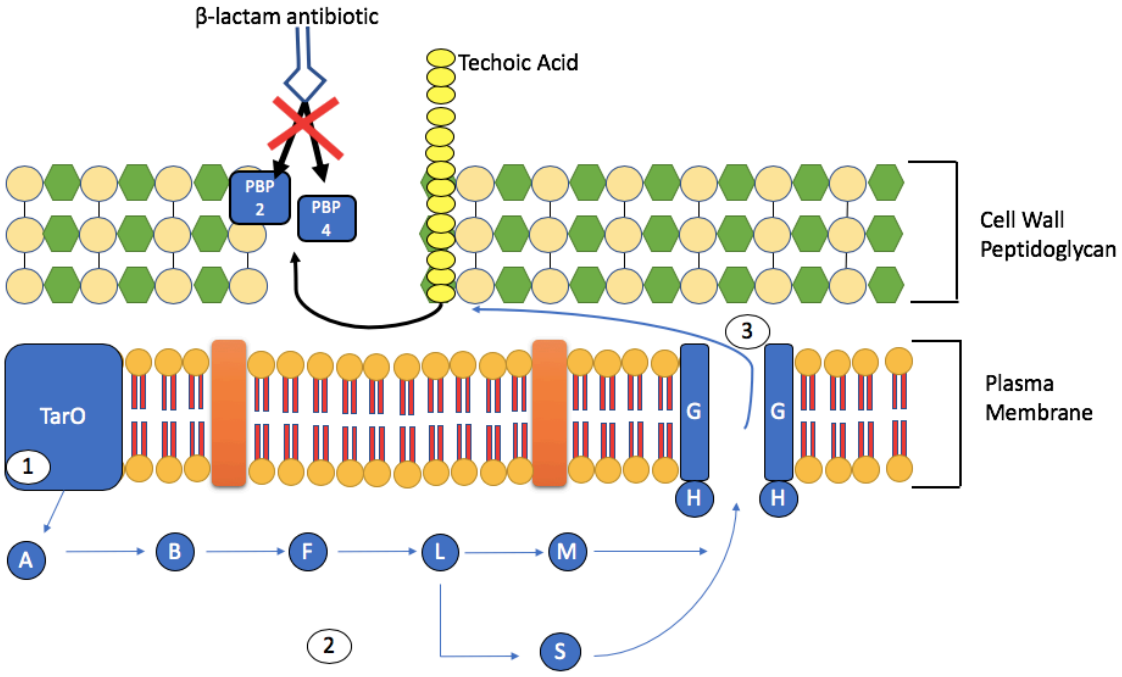

Figure 3. Schematic Diagram representing β-lactamase degradation of β-lactams in Methicillin-Resistant *Staphylococcus Aureus*. The β-lactam binds to the plasma membrane receptor, blaR1(1). The blaZ repressors (blaI and blaR) are degraded (2), facilitating blaZ activation (3). β-lactamase is then synthesised (4) and enzymatically cleaves the β-lactam (5) (Hao *et al.*, 2012).

β-lactamase inhibitors such as clavulanic acid have been developed to inhibit the β-lactamase enzyme in *S. aureus* infections (Ba *et al.*, 2015). While not powerful enough to kill MRSA cells on its own, β-lactamase inhibitors have been shown to work collaboratively with β-lactams to kill MRSA (Ba *et al.*, 2015). This action is highly dependent on the MRSA strain that is present. It has been proposed that this is due to variations in PBP structures amongst different MRSA strains (Hryniewicz & Garbacz, 2017). Furthermore, different combinations of antibiotics with β-lactamase inhibitors are used to treat this. Therefore, it is difficult to attribute β-lactamase inhibition to β -lactam activity or to other antibiotics administered during treatment (Henson 2017).

Wall techoic acids (WTAs) are macromolecules attached to the cell wall peptidoglycan, which can influence cell functions such as cell division (Brown *et al.*, 2012). WTAs influence on cell function have recently been implicated in MRSA β-lactam resistance (Foxley *et al.*, 2017).

As depicted in Figure 3, WTAs play a role in resistance by regulating the trafficking of PBP2 and 4 (Farha *et al.*, 2013). Tar enzymes are involved in WTA production and their expression has been associated with β-lactam resistance. For example, inhibition of TarS brought about MRSA sensitization to β-lactams, and not to other antibiotics, suggesting that TarS expression is a β-lactam-specific resistance mechanism (Sobhanifar *et al.*, 2016). Although this finding has yet to be confirmed in human patients, it may act to strengthen first-round MRSA treatment (Foxley *et al.*, 2017). One difficulty in identifying a valid therapeutic target is that Tar gene expression varies across different MRSA strains. For example, the enzyme TarS has been found amongst nearly all MRSA strains while TarM, a similar functioning enzyme, has only been found in only a few strains (Sobhanifar *et al.*, 2016). Unfortunately, these two enzymes have very different structures which makes it difficult to target both of them with one single drug (Sobhanifar *et al.*, 2016). Interestingly, inhibition of the early stages of MRSA cell wall biosynthesis is more likely to make MRSA bacteria respond to β-lactams than inhibition of enzymes in the later stages of bacterial cell biosynthesis (Campbell *et al.*, 2012). This was demonstrated through the use of the TarG inhibitor targocil. The effects were distinct from TarO inhibition in MRSA bacteria (Campbell *et al.*, 2012). However, it raises questions as to how effective the treatment would be depending on when the therapeutic is administered. Nevertheless, development of WTA inhibitor drugs could prove promising and effective when used in combination with β-lactams as WTA inhibitor administration could re-sensitize MRSA bacteria to β-lactam therapy.

Conclusion

MRSA has become resistant to β-lactam antibiotics through various mechanisms due to selective pressure and the inappropriate use of antibiotics. Resistance mechanisms, including MecA, β-lactamase, and the WTAs, have brought about great challenges in finding effective treatments for MRSA infections. While certain new drug targets have been identified, several obstacles remain in order to combat the future evolution of MRSA. The current evidence suggests it is optimal to develop synergistic drugs that both hinders MRSA resistance mechanisms and kill the bacteria. This would greatly slow down further development of MRSA resistance to therapeutics and could facilitate the reintroduction of previously effective β-lactam therapeutics.

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The potential of the curcumin in preventing the onset and progression of Alzheimer's disease

Daisy Stewart

Introduction

Alzheimer's disease is a progressive neurodegenerative disorder which typically develops past the age of 65 (Alzheimer's Association, 2016), and was first described by Alois Alzheimer in 1907 (Alzheimer, 1907). There are approximately 50 million people worldwide with dementia, about two thirds of whom have Alzheimer's (Alzheimer's Disease International, 2018), and this number is growing with the ageing population. The disease starts in the hippocampus and medial temporal lobe where it causes memory loss, and spreads across the whole cerebral cortex affecting cognitive processes such as language and problem solving (Alzheimer's Disease International, 2018). This can lead to mood changes, confusion and disorientation, which worsen the sufferer's quality of life (Alzheimer's Disease International, 2018).

Alzheimer's pathology is characterised by abnormal protein deposits: aggregations of amyloid-beta (Aβ) protein known as amyloid plaques, and neurofibrillary tangles (NFT) formed from the protein tau (Hardy, 2006). These are believed to cause the subsequent loss of neural tissue through mechanisms which are not fully understood, despite advances in recent research (Sanabria-Castro et al., 2017). Consequently, there is no cure for Alzheimer's and current prescribed drug treatments, acetylcholinesterase inhibitors and NMDA receptor antagonists, only reduce or delay some symptoms (Alzheimer's Disease International, 2018). There is therefore an urgent need for an effective cure or preventative measure for the disease.

Utilising the neuroprotective powers of natural compounds, such as curcumin, may fulfil this requirement. Curcumin derives from the roots of the *Curcuma longa* plant and is present in the spice turmeric. Its anti-inflammatory and anti-oxidant properties are well-characterised and evidence for its neuroprotective effects is growing, particularly with relevance to Alzheimer's disease (Sharma et al., 2005). This review will explore the roles of curcumin in preventing or reversing Alzheimer's pathogenesis and the clinical application of these, including the current stage of development of curcumin-based therapies and barriers to drug design.

Discussion

Anti-Amyloid Activity

Curcumin was shown to reduce the number of amyloid plaques in the hippocampi of a mouse model of Alzheimer's (McClure et al., 2017). Their cognitive function was also significantly improved which highlights the clinical relevance of this effect. This is in line with evidence that curcumin disaggregates fibrillar Aβ protein in cultured cells *in vitro* (Yang et al., 2005). It is unclear whether these effects would be maintained over long-term treatment, due to the short time span of both studies. However, in addition to breaking down plaques, curcumin was also able to block their formation and may therefore play a role in preventing disease onset. Its ability to directly bind Aβ appears central to this; assaying molecularly similar compounds will help validate this finding and enhance treatment development.

Anti-Inflammatory Activity

Inflammation is a clear feature of Alzheimer's, indicated by high levels of pro-inflammatory molecules like cytokines and complement factors in the brain. These activate immune cells like microglia, which release further inflammatory factors (Akiyama et al., 2000). Increased numbers of microglia are present in disease legions compared to the healthy brain, particularly surrounding amyloid plaques (Uchihara et al., 1997). It appears that they are activated by pro-inflammatory mediators secreted by the plaques but are unable to remove them, remaining in a pro-inflammatory state (Lee and Landreth, 2010). Chronic inflammation in the brain is highly damaging and contributes to functional deficits and death in neurons.

Epidemiological studies suggest that non-steroidal anti-inflammatory drugs (NSAIDs) not only slow the progression of Alzheimer's but reduce the risk of developing the disease, which supports the notion that inflammation plays a causal role in its onset. The most common NSAID, Ibuprofen, cleared Aβ deposits and reduced the numbers of microglia around these in the brains of mice (Lim et al., 2000). However, the amount of ibuprofen used in this study exceeded the determined safe dose for humans, so it cannot be assumed that the same effect would occur in patients.

Curcumin is a well-known anti-inflammatory agent which has proven neuroprotective effects (Ghosh et al., 2015). Phosphatidylserine (PS) nanoparticles used to deliver

curcumin to macrophages (the body's equivalent of microglia) reduced pro-inflammatory cytokine expression and lipid uptake (Wang et al., 2016). This occurred at the level of mRNA, which is produced from DNA, suggesting that curcumin modulates gene expression in these cells. The reduction in intracellular lipids is significant as certain types of lipids such as long-chain saturated fatty acids have been demonstrated to stimulate the inflammatory action of both macrophages and microglia (Håversen et al., 2009, Wang et al., 2012), and excessive lipid accumulation can trigger metabolic abnormalities in macrophages, as seen in high-fat diet-induced obesity (Lumeng et al., 2007). However, the type of lipid is important as unsaturated fatty acids are not pro-inflammatory (Lee et al., 2001). Nonetheless, PS nanoparticles represent an effective strategy to deliver curcumin to cells which could be employed in drug development.

The results of this *in vitro* research are in line with a study in mice in which treatment of curcumin for 3 days reduced cytokine secretion in macrophages (Ma et al., 2017). A key mechanism by which this occurred was the downregulation of microRNA via the PI3K-Akt signalling pathway, which promotes cell proliferation via the protein mTOR (Yu et al., 2016). Macrophages may behave differently to microglia, but this study is supported by the finding that curcumin has similar effects on microglial activity (Cianciulli et al., 2016), including changes to their metabolism and also occurring via the PI3K-Akt pathway. This emphasises the importance of this pathway in curcumin's actions and it may be pivotal for understanding curcumin's role in Alzheimer's and the mechanisms underlying inflammatory diseases.

Curcumin's anti-inflammatory effects involve additional molecular targets including chemoattractants, which it downregulates to prevent recruitment of immune cells to disease legions (Giri et al., 2004). It may be important to ensure that there are no large structural changes to a curcumin-based drug in order to maintain these diverse roles and maximise its efficacy.

Anti-Oxidant Activity

Oxidative stress, a process closely related to inflammation, is another factor in the formation of Aβ plaques and NFTs and is induced by the plaques themselves (Butterfield et al., 2013). The brains of Alzheimer's patients exhibit lesions associated with exposure to oxidising agents like free radicals, and antioxidants have been shown to suppress neuronal death in a mouse model of Alzheimer's (Kwon et al., 2016). Epidemiological studies suggest a higher dietary intake of antioxidants vitamins C and E could reduce the risk of developing Alzheimer's (Engelhart et al., 2002), although other lifestyle factors were not controlled and may have impacted the results of the study. Contrary to this, Morris et al. in 2002 reported no significant relationship between vitamin C intake and the development of Alzheimer's, perhaps indicating a larger contribution of other lifestyle factors to disease risk (Morris et al., 2002).

Curcumin is a potent antioxidant and effectively decreases the levels of free radicals *in vitro* (Ak and Gülçin, 2008). Similar effects have been reported *in vivo,* as curcumin-treated mice upregulate several antioxidant enzymes via the transcription factor Nrf2 (Shen et al., 2006). This includes heme oxygenase-1 which protects vascular endothelial cells and maintains the blood-brain barrier (BBB) (Wang et al., 2013), and has also been demonstrated to prevent neuronal cell death via carbon monoxide production (Hettiarachchi et al., 2014).

Further mechanisms by which curcumin prevents oxidative stress have been demonstrated to include the downregulation of pro-oxidant enzyme superoxide dismutase and the reversal of lipid peroxidation, the oxidative degradation of lipids (Li et al., 2017). This demonstrates a link to the aforementioned ability of curcumin to reduce microglial lipid uptake. In addition, lipid peroxidation end products can induce abnormal cross-links on proteins which impair their function (Negre-Salvayre et al., 2008). They are a feature of Alzheimer's and may be involved in the formation of amyloid plaques and NFTs (Sayre et al., 1997). Protein dysfunction is also strongly associated with cell death and inflammation (Wang et al., 2014).

Translation to Treatment

The primary obstacle in the development of curcumin-based drugs is that conventional methods are ineffective in delivering the free form to target tissues. With oral delivery it is not well absorbed across the intestine, and intravenous injection is only slightly more effective because its absorption across the BBB is poor (Pardridge, 2009). There is therefore extensive ongoing research into alternative methods of delivery.

An aerosol, inhalable form of curcumin delivered the compound to the brains of mice by bypassing the BBB through the olfactory epithelium system (McClure et al., 2017). This method achieved an even distribution of curcumin across the brain in a shorter time span than oral delivery with no registered toxicity, which appeared to be due to the avoidance of first-pass liver metabolism (McClure et al., 2015). Furthermore, this treatment had positive effects on disease pathology and symptoms; the numbers of Aβ deposits were reduced and cognitive function was measurably improved.

Treatment with free curcumin is further limited by its amphiphilic nature, as it contains both hydrophilic (water-soluble) and hydrophobic (water-insoluble) parts. A form that is sufficiently lipid-soluble to cross the intestine and BBB

but hydrophilic enough to travel by blood circulation is required. In one study, entrapment of curcumin in modified linoleic acid micelles (lipid particles) increased its plasma solubility approximately $1.87x10^8$ times that of free curcumin (Song et al., 2014). The copolymer used in the micelles was confirmed as safe for intravenous injection. Additionally, there was controlled release of curcumin in target tissues, which proved faster than with unmodified micelles. This ensured that it reached the brain, which a higher plasma concentration alone may not achieve because curcumin is rapidly removed from the blood and metabolised.

Furthermore, the low cost and natural abundance of curcumin make it a highly desirable treatment. It has undergone extensive toxicological screening and pre-clinical testing in animals to ensure its biological safety. Adverse effects have not been seen in clinical trials, even with doses up to 8000 mg/day (Sharma et al., 2005), which is particularly important given that it is a hormetic and demonstrates an opposite response at a high concentration. However, intracellular curcumin concentrations were likely to have been limited by the aforementioned reasons, so it is vital to ensure that changes in dosing methodology do not produce adverse effects. Furthermore, the long-term effects of curcumin treatment are largely unknown due to the short time span of most studies and must be well characterised before its clinical use.

Conclusion

Inflammation and oxidative stress are primary mechanisms in Alzheimer's pathology and involve the Aβ plaques and NFTs which are characteristic of the disease. These appear to trigger neural tissue loss both directly and indirectly through various mechanisms, including the activation of pro-inflammatory microglia. Expanding evidence from both cell and animal research suggests that curcumin may prevent or slow Alzheimer's pathogenesis, through diverse mechanisms which appear mainly anti-inflammatory and anti-oxidant. These include the removal of amyloid plaques and reduced microglial activity through changes in gene expression which may rely on the PI3/Akt pathway.

A more precise understanding of the mechanisms of curcumin's roles in Alzheimer's, including the identification of other signalling pathways and binding partners involved, is key to effectively utilise it as a treatment. Further research into Alzheimer's pathology, particularly factors in the formation of abnormal protein deposits, will aid drug design. Curcumin's properties pose several barriers in delivering it to target cells, but research into alternative delivery strategies has so far proven promising. Utilising these methods to replicate positive *in vitro* studies *in vivo* will ascertain whether the same results may be replicable in patients, before they are translated to clinical trials.

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UgSJ Undergraduate Science Journal University of Exeter

The extended phenotype of tent-making bats

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Abstract

The extended phenotype describes the concept whereby organisms affect their environment as an expression of their genes. Objects they build therefore become extensions of their phenotype. The leaf tents built by some bat species are an extended phenotype. This research focuses on a population of tent-making bat species on Utila, Honduras, describing the morphology of the tents. The study explores why bats abandon incomplete tents, and if plant species or tent occupancy affects tent condition. 306 tents were measured and the data analysed with two-sample t-tests. Crown width was larger on incomplete tents, suggesting that a mistake in tent construction led to tent abandonment. Tent condition varied with plant species, possibly indicating bat movement or differing rates of leaf decay. Condition was higher in occupied tents, likely due to tents in poor condition being unable to support roosting bats. This research explores this extended phenotype, highlighting areas for future research.

Introduction

The extended phenotype is the term that explains the concept by which genes do not just affect the phenotype of an organism (its outward appearance), but can, via behaviours, affect the environment the organism lives in (Dawkins, 1982). Architectural structures that an organism builds, such as spider webs (Nakata, 2012) or beaver dams (Dawkins, 1982), can then be considered an *extension* of the organism's phenotype. Another example of this is found in bats. 22 bat species are known to chew leaves to build structures known as 'tents' (Rodríguez-Herrera *et al*, 2007). These tents are thought to be important in the mating systems of the bats (Kunz & McCracken, 1996; Tan *et al*, 1997) and play a key role in avoidance of predators, extreme weather conditions (Foster & Timm, 1976) and parasite infections (Lewis, 1995). Although extended phenotypes in bats are known to exist (Dechmann & Kerth, 2008) , studying tent-making as an extended phenotype has not been specifically examined.

Two species of tent-making bats are found on the island of Útila, Honduras (16.0950° N, 86.9276° W), and are both in the genus *Artibeus* (family: Phyllostomidae). *A. phaeotis* is a small, fruit-eating bat with a body length of 51-60mm (Timm, 1985). *A. jamaicensis* is much larger, with a body length of 78-89mm (Ortega & Castro-Arellano, 2001). This research documents the characteristics of the tents found in this relatively understudied island population, as well as explore variation within this extended phenotype. The report will focus on three questions about tent building: why bats leave tents unfinished, if plant species affects tent condition, and if there is a difference in condition between vacant and occupied tents.

Expanding our knowledge of tent-making behaviour in bats will assist our understanding of extended phenotypes, and how they can vary as a result of differing factors.

Hypotheses and predictions

Complete and incomplete tents

Given that tent-making is energetically costly for bats (Timm *et al*, 1991) it is likely that only sizeable disturbances would cause a bat to leave a tent unfinished. However, it is possible for the bat to return and complete the tent once the disturbance has passed. This study therefore hypothesises that bats are capable of making mistakes in tent construction, leading to tents being abandoned when incomplete. If this is the case, a predicted significant difference will be found in one, or many, tent characteristics between complete and incomplete tents.

Differing plant species

An observed difference in tent condition between plant species may indicate a difference in the rate of leaf decomposition. This has been shown in previous tent-making bat studies, where some tents in large *Sabal* palms can last up to 9 months (Kunz & McCracken, 1996), whereas others made of *Heliconia* sp. last for less than a month (Chaverri & Kunz, 2006). This may have wider impacts on tent usage in different plants.

Occupied and vacant tents

As palm leaves age, their petioles weaken. This is likely to restrict how long a bat can use a tent for. Bats have also been observed to use nearby vacant tents as escape shelters should they be disturbed (Boinski & Timm, 1985). Therefore, this study predicts occupied tents will be in better condition than vacant tents, with some vacant tents in good condition for use as escape shelters.

Figure 1. A) A palmate umbrella tent in hardwood forest. B) A photo taken below the tent showing bats roosting inside. C) Digital enhancement of the image identifies the species as *A. phaeotis*.

Methods

The study was conducted on the two species of tent-making bat found on Útila from mid-June to late-July, 2018. 8 field locations, covering 7 habitat types, were surveyed for tents and all tents found below 4.5m above the ground were measured. Approximately an hour and a half search time was allocated for each day of surveying, with an average of 11 tents being measured an hour. Once a tent was located, the GPS, plant species, number of leaves on the plant and tent-type were recorded. Plant species was determined via the presence or absence of petiole barbs. Those without barbs were classed as *Sabal* spp A, and those with, Sabal spp B. To minimise disturbance to the bats, tents were then tested for occupation via a camera phone mounted on a 3m long selfie-stick held below the tent. A photo was taken (see Figure 1), checked, and if the tent was occupied, the species and number of bats were recorded. If the tent was unoccupied, the base of the petiole circumference, leaf blade length, height above the ground and crown width were measured.

Light intensity both inside and outside the tent were then measured using a XT-XINTE BSIDE ELM02 Digital Light Meter. Should a tent be occupied, it was visited at a later date, when vacant, to take all the measurements required. All measured tents were marked to prevent pseudo-replication, and the GPS location was mapped onto a 87.86% accurate landcover map of Útila (Higgins, Unpublished) to determine the habitat type. Finally, a condition score was given to the tent based on 10 observed signs of tent aging, with 10 being the score of the tent in the worst condition.

A total of 306 tents were sampled from 95 different plants, the data from which was then analysed with R. Two-sample *t*-tests (or rank sum tests if data was non-parametric) were used to compare characteristics of complete and incomplete tents, condition scores between the two plant species used for tents, and condition scores between occupied and vacant tents.

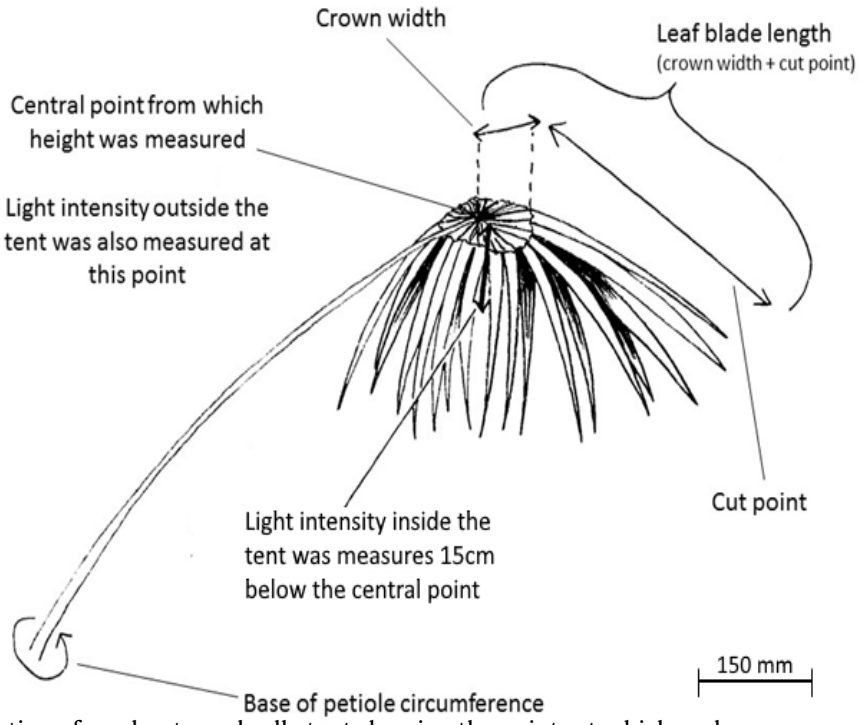

Figure 2. Illustration of a palmate umbrella tent showing the points at which each measurement was taken.

Results

The only tent shape found on the island were palmate umbrella tents, as shown in figure 2. A summary of the completed tent characteristics measured on Útila can be found in Table 1.

There was no difference between completed and incomplete tents in tent characteristics such as condition (Student *t*-test: $t_{277} = 0.544$, $P = 0.587$; square-root transformed data), leaf blade length (Wilcoxon rank sum test: $W_{194,22} = 1665.5$, $P = 0.092$), height above ground (Welch's *t*-test: $t_{28.206} = -0.485$, $P = 0.631$) or cut point (Wilcoxon rank sum test: $W_{179,15} = 1112$, $P = 0.271$). However, incomplete tents had larger crown widths than completed tents (Welch's *t*-test: $t_{16.718} = -5.943$, $P < 0.001$; log transformed data; Fig. 3a).

Plant species affected the condition of tents, with *Sabal* spp. B plants containing higher quality tents than *Sabal* spp. A (Wilcoxon rank sum test: $W_{238,15} = 2420.5$, $P = 0.018$; Fig. 3b). Occupied tents were in better condition than vacant tents (Wilcoxon rank sum test: $W_{3,250} = 129.5$, $P = 0.046$; Fig. 3c).

Discussion

Complete and incomplete tents

The observation of so few incomplete tents provides evidence that it is costly to leave a tent unfinished. The significant difference found between complete and incomplete tents in crown width follows the predicted observation and could provide evidence that mistakes in tent construction do occur. Should a bat chew a crown width that is too large, the bat is likely to abandon the tent unfinished. Crown width may therefore control an important factor in successful tent construction.

Differing plant species

Tents constructed using leaves of *Sabal* spp B were in better condition than *Sabal* spp A. It could be that *Sabal* spp B decays at a slower rate than *Sabal* spp A, and could therefore be a better plant species in which to build tents. Another explanation is that the tents in *Sabal* spp B had only recently been made.

Table 1. Mean averages (to 1 decimal place) and ranges of the tent characteristics observed on Útila (completed tents only). ±SE

Tent Characteristic	$\bar{x}$	n	Range
Petiole base circumference (mm)	65.6 ± 0.9	232	40 – 108
Condition score	4.9 ± 0.1	253	2 – 10
Leaf blade length (mm)	568.1 ± 6.4	212	163 – 850
Crown width (mm)	76.8 ± 1.8	199	38 – 159
Cut point (mm)	490.4 ± 6.3	180	326 – 740
Number of tents per plant	3.3 ± 0.4	88	1 – 19
Height above the ground (m)	1.8 ± 0.1	264	0.03 – 4.15
Proportion of light intensity inside the tent (Lux)	0.1 ± 0.0	72	0.01 – 0.83

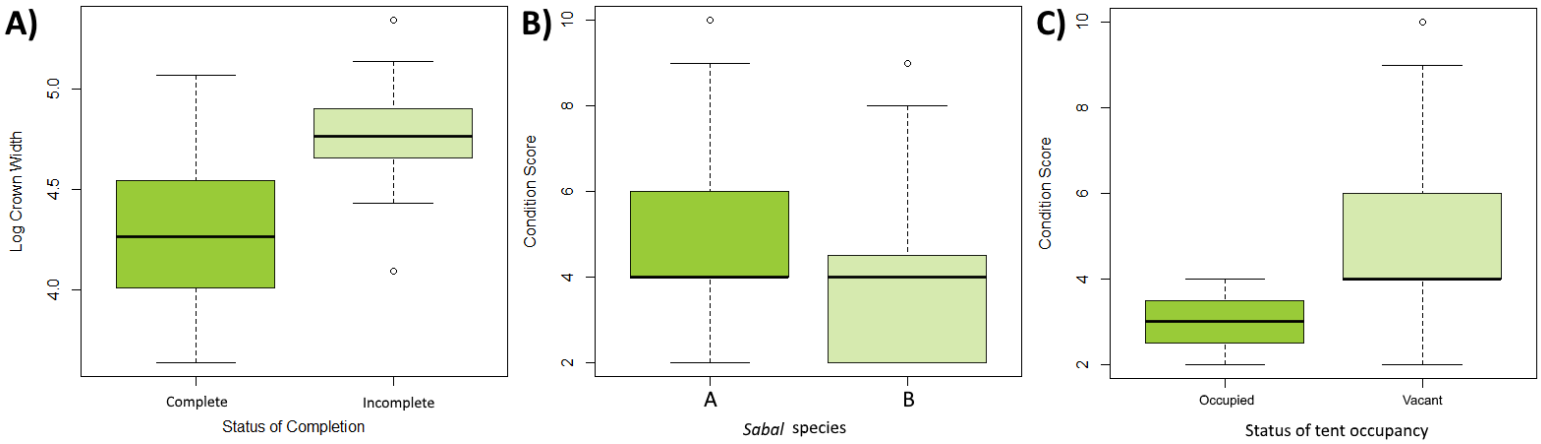

Figure 3. A) Comparing the log-transformed crown width between complete (n=198)and incomplete tents (n=15). B) Condition score differences between *Sabal* spp. A (n=238) and spp. B (n=15). C) Condition score differences between occupied tents (n=3) and vacant tents (n=250).

The abundance and diversity of Phyllostomid bat species, such as *A.jamaicensis* and *A.phaeotis*, has been recognised as an indicator of disturbance (Medellín *et al*, 2000). Given that, during the study, lots of building development and disturbance was occurring in easily accessible areas of *Sabal* spp A (M. J. Inston, pers. obs.), it may be that the bats were beginning to disperse into less easily accessible areas for humans. *Sabal* spp B appeared to be more present in less accessible areas, and so this may be the reason for the newer tents being found there. Should this be the case, plant use in tent construction could be developed as an indicator of disturbance level in the environment.

Occupied and vacant tents

As predicted, occupied tents were in better condition than vacant tents. As leaves age, their petiole becomes weaker and can hold fewer bats – therefore, only tents in better condition can feasibly be occupied. In addition, there were many more vacant tents than occupied ones. The use of these vacant tents as escape shelters was observed, as described by Boinski & Timm, 1985, with the bats flying to nearby tents for safety when accidentally disturbed.

Conclusion

This study is the one of the first conducted on the tent-making bats of Útila, and provides an important insight into how they use their environment and respond to change.

Future studies could examine which potential factors crown width controls, and thus why making it too large causes tents to be discarded. Research could also be done into whether *Sabal* spp B decays at a faster rate, and if it does not, question whether bats are then dispersing into more inaccessible areas to flee human disturbance.

This study is also the first to examine tent-making as an extended phenotype in detail. As Laland's 2004 paper stresses, extended phenotype is not always a product of genes (Laland, 2004). Organisms are also capable of learning to create structures. Given that social learning is known to occur in bat species (Spanjer Wright *et al*, 2011; Ratcliffe & ter Hofstede, 2005), exploring whether or not bats learn to make tents from each other is a fascinating area for future research.

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UgSJ Undergraduate Science Journal University of Exeter

Direct imaging of Exoplanet and Circumstellar Disks

Yee Shan Lau

The aim of this project is to search for companions and debris disks among one of the largest data sets available for imaging exoplanets by W.M. Keck Observatory. Discoveries have been made in this survey, in particular, the result of a possible companion of HIP 16095 based on K′ band observations is presented in this report. This candidate is ≈ 3.3 arc second (≈ 340 AU) away from HIP16095. Follow-up observations and analysis are required to confirm if this object is gravitationally bounded by HIP16095.

Introduction

Extrasolar planets, known as exoplanets, are planets outside the Solar System. Prior to March 2019, more than 3800 exoplanets has been discovered by various methods of which 3000 are transiting planets. Only 44 planets are firstly detected by direct imaging (*NASA exoplanet archive planet counts*, 2019).

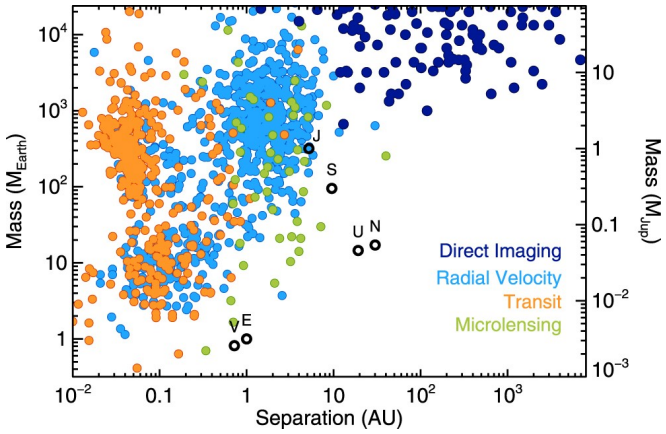

Figure 1. Summary of the discovery of exoplanets by Bowler (2016). Colours represent different methods for finding exoplanets. Meanwhile, circles with letters are planets in the solar system.

Figure 1 indicates the exoplanets found by different methods. Separations between planets discovered by various methods has hinted that there are intrinsic biases originating from the method. Transit is by far the most successful method as more than 3000 exoplanets are transiting. Transit is a phenomenon when the planet periodically passes in front of the star and reduces the apparent brightness of its host, which results as a dip in the light curve. Transiting planets can be followed up by radial velocity. Radial velocity, also known as Doppler Spectroscopy, measure the velocity of star along the line of sight by high-resolution spectroscopy using the Doppler effect. The gravitational pull of the companion causes a periodic variation of the star′s radial velocity. These methods are favourable for searching edge-on, close-in planets with short orbital period.

However, these approaches are not well-suited for detailed characterisation of planets around stars. Doppler spectroscopy measures orbital period, eccentricity and mass without revealing other physical information of the companion such as radius and atmospheric composition. Transit provides information on the radius of the companion derived from the transit′s depth = $R^2_{companion} / R^2_{star}$ (R being the radius). Although the atmospheric composition can be obtained through transit spectroscopy, the sample size and data quality are strongly limited by the low transit probability per star and stellar photon noise.

In contrast to these methods, high contrast imaging enables direct spectroscopic measurements of exoplanets. Multiple observations allow us to characterise orbital parameters and its atmosphere. Higher resolution of spectra can be obtained, as physically isolating the planet light from starlight will yield a higher signal-to-noise ratio with combined light observations. High-contrast imaging also offers us a window for directly observing the planet formation process at various systems and timescales. Direct detection of photons emitted from planets provides valuable information on initial conditions, structures, atmospheric conditions and physical properties of the planets. Indirect detection methods and direct imaging provide complementary measurements as mass cannot be determined without Doppler spectroscopy or precise measurements of positions and movement on the sky (astrometry).

Our aim is to search for exoplanets and circumstellar disks in one of the largest digital data-set available for imaging exoplanet obtained from W.M. Keck Observatory. This data-set consists of more than 300 stars, mostly A and F-type with mid-infrared excesses from the NASA WISE mission. Most stars are classified under spectral classes using letters O, B, A, F, G, K, and M, a sequence from the

hottest (O type) to the coolest (M type). Other than searching existence of disks and planets, we would like to develop algorithms for further subtraction of quasi-static speckle noise induced by the system, establishing the link between the existence of disks and planets, devising new algorithms to search for faint candidate companions, follow up on potential candidates and calculate the sensitivity of this survey. In the following sections, principle of direct imaging, reduction of the data-set, initial results and future plans of this project are described in this report.

Principle of Direct imaging

Direct imaging has been playing an important role in probing orbital separations beyond ≈ 10 Astronomical Units (1 AU ~ 150 million Km) and mass ≥ 1 Jupiter Mass (M_{Jup}) as it requires planets at large orbital separation. Discovery of wide-separated planets motivates the development of theories for planet-formation and migration, also inspiring the enhancement of theories for the origin of the giant planets which is affected by multiple mechanics at different timescales and orbital separations. An increase in sample size will enable us to answer open questions and test our current planet formation theory.

Results produced in the past 20 years have suggested a promising future development of direct imaging. We are transitioning to extreme adaptive optics system, for instance, in James Webb Space Telescope and thirty-metres class ground-based telescope. The extreme adaptive optics system (ExAO) refers to adaptive optics system (AO) which is designed for a high degree of wave-front correction on relatively bright natural guide stars. These are stars which can serve as point sources for reference. ExAO runs faster and have more actuators and sensors than the AO. In future, combinations of methods will provide a complete picture for studying planetary formation and dynamics.

High-contrast imaging is a direct method for searching planets which requires stabilisation of the starlight, application of coronagraphic mask and correction of residual starlight. Below is a brief description of the mechanism of adaptive optics.

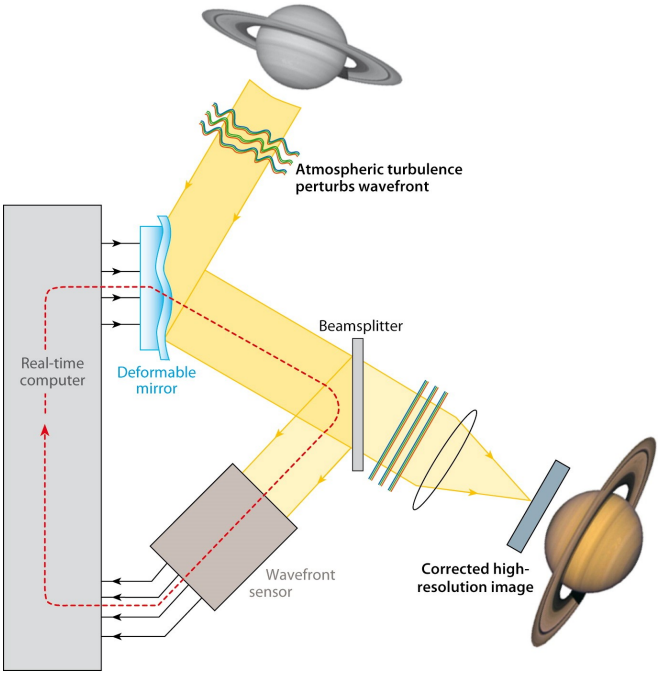

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Figure 2. A schematic diagram of principle of Adaptive Optics from Davies and Kasper (2012). The main components of the adaptive optic system (wave-front sensor, wave-front corrector and a real-time control system) are shown.

The adaptive optics will regulate the optical path variation by measuring deviations of the wave-front with the wave-front sensor (WFS). WFS measures the optical aberrations induced by atmospheric turbulence and optics. Then, the corrections will be calculated by the computer and applied to the deformable mirror (DM). Deformable mirror is a small mirror whose shape can be adjusted to rectify changing optical aberrations. The process depicted in Figure 2 is repeated several hundred times per second in a real-time control system to comply with the temporal bandwidth requirement set by coherence time. The coherence time (τ_0) is the time interval up to which optical path variations differ by less than one radian root-mean-square phase aberration from each other. This defines the required adaptive optics temporal correction bandwidth, which is typically a few milliseconds at a visible wavelength. DM commands are applied to follow the continuously variation of the atmospheric wave-front distortion.

After stabilising the starlight and correcting the wave-front aberration, a coronagraph is required for reducing the amount of incoming light on our detector. It is a telescopic instrument designed to suppress starlight by using optical masks in pupil and focal planes. The final element of high

contrast imaging is to correct any residual starlight by observing strategies and post-processing algorithms.
High contrast imaging is challenging for several reasons: wave-front aberration caused by atmospheric turbulence, massive difference in contrast between the host star and its companions (from 10^{-3} to 10^{-10}) and small angular separation between them. In order for direct imaging to work, we need adaptive optics for stabilising the starlight, a coronagraphic mask to block most of the starlight and correct any residual light by post-processing and observation technique. Moreover, with the combination of wave-front control and coronagraph, we still cannot remove the quasi-static noise pattern introduced from the telescope and instruments (Marois et al., 2005; Hinkley et al., 2007). These speckles produced by aberrations of instruments mimic the presence of point source because they are comparable in angular size and brightness. This indicates the importance of optimising observing techniques and post-processing techniques.

Methods

Python is our main tool for data analysis and image processing, more specifically, "Vortex Imaging Processing (VIP)" will play the main role in this project. This is a package developed for angular, reference star and spectral differential imaging for exoplanet and disk detection through high-contrast imaging by Gonzalez et al. (2017). A pipeline has been developed for image-pre-processing in the K′ band. Spectral bands are wavelength bands of light, a band filter shows the part of the stars spectrum that the observer is interested in. For example, H and K bands are filters which central wavelengths are 1.633 μm and 2.196 μm respectively, this is in the infrared region. J and H band (also in infrared) processing is similar to K′. However, L band processing will require additional steps because L band data suffer from the thermal emission of the background.

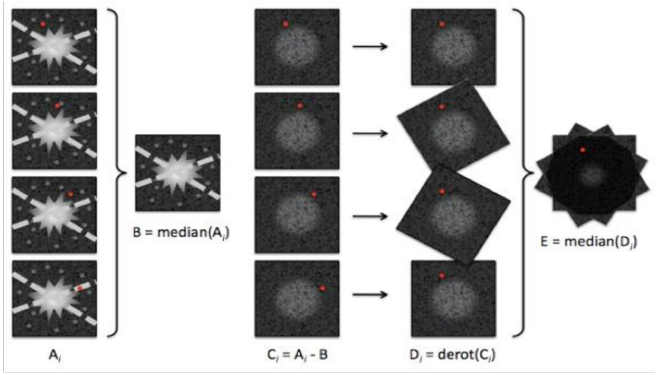
Image Reduction and Processing

Image pre-processing is essential as this improves the image data by the suppression of unwanted distortions, which may enhance some important features for further processing. Noises and defects are corrected by the application of flat fields (uniformly illuminated field) and dark frames (images captured with the sensor in the dark) so uneven illumination on the surface of imaging sensor, illumination of dust particles on the telescope and thermal noise can be corrected. Removal of dead pixels can be done by a dead pixel map created from a mask, which will replace the bad pixels with the median of the nearest neighbour pixels inside a square of variable size.

A data cube, or a sequence of stacked images in a Flexible Image Transport System (FITS) file, is required for post-processing. An image cube is created by aligning the transmission spots at the centre of the coronagraph and registered by fitting a 2D-Gaussian. Then, the data cube is analysed by several algorithms to produce final images and sensitivity limit of the observation. The principles of the algorithms we used for image reduction are explained in the following section.

Angular Differential Imaging

Angular Differential Imaging (ADI) (Marois et al. (2005)) is a powerful observation and post-processing technique for high contrast imaging which is the observing technique and one of the post-processing algorithm applied in this survey.



(Marois et al. 2006)

Figure 3. Visualisation for the mechanism of angular differential imaging based on Marois et al. (2005).

The median of all the images is subtracted from each individual image. For a sequence

$$I_1(t_1,\theta_1),\ I_2(t_2,\theta_2),\ I_3(t_3,\theta_3),\ ...,\ I_n(t_n,\theta_n) \qquad (1)$$

of n reduced and registered images, where t_i is the mean time of exposure, and θ_i is the Field of View (FOV) orientation at the time t_i; the first reference subtraction is

$$I_i^D = I_i - \text{median}(I_1, I_2, I_3, ..., I_n) \qquad (2)$$

An optimal reference point-spread-function is then obtained for each image by median combination of four images that show at least a 1.5 full width at half maximum (FWHM) FOV rotation difference. FWHM is a width on a curve of functions given by the distance between points on the curve at which the function reaches half its maximum value. The point-spread function (PSF) describes the two-dimensional distribution of light for a point source. In this case, the linear quasi-static noise speckles have the correlation timescale of $\tau_{speck} \geq 2\tau$ (the time interval between the image and its reference) is removed.

$$I_i^{ADI} = I_i^D - a \times \text{median}(I_{i-b}^D, I_{i-b-1}^D, I_{i+c}^D, I_{i+c+1}^D) \quad (3)$$

where a is the normalisation factor to minimise the noise inside the annulus (ring-shaped area), and the number of images required to get at least 1.5 FWHM FOV rotation difference between I_i and the images required before and after I_i are b and c. Images are then rotated to align the FOV of the first image. Finally, the median is taken over all differences. The final step is

$$I_F^{ADI} = \text{median}[I_1^{ADI}, \text{rot}(I_2^{ADI}, \Delta\theta_{1-2}), I_3^{ADI}, \Delta\theta_{1-3}), ..., \text{rot}(I_n^{ADI}, \Delta\theta_{1-n})] \quad (4)$$

The second algorithm for post-processing ADI data is Local Low rank plus Sparse plus Gaussian noise components (LLSG). LLSG is local three-term decomposition of an ADI image sequence (Mawet et al. (2016)). Principal component analysis is the third algorithm we used for post-processing. This method has been extensively applied in astronomy to reduce large data sets. In this case, principal components are used to reduce the dimensionality of the reference set, using eigenimages instead of reference PSF. The algorithm we are using is based on Soummer et al. (2012) and Amara and Quanz (2012).

Aperture photometry

Aperture photometry has been done in 2 passbands to estimate colour of this object. Passband refers to a range of wavelengths which can pass through the filter. In astronomy, the colour of an object means the difference of magnitudes of the same object in different passbands. The coronagraphs for NIRC2 (near infra-red imager) at Keck are not completely opaque. The transmission at the centre of NIRC2 600 milliarcsecond coronagraph in H is 0.1% and in the K_s band is 0.22 % measured by Bowler et al. (2014). The only information available is the contrast which can be transformed to apparent magnitude once the flux of the star is acquired. On top of being calculated from the PSF, the flux can be estimated by the central peak of the mask. Since the apparent magnitude of HIP16095 is known, the magnitude of the candidate can be calculated by its contrast. Every science frame is checked to confirmed that there is no residual starlight overlapping our target. Co-add (adding the images together) and integration time have to be the same in the image series, so average can be taken. The magnitude of the possible companion is obtained from the following equations

$$\frac{F_b}{F_{star}} = 10^{0.4\times(m_{star}-m_b)} \quad (5)$$

$$F_{star} = \frac{F_a}{T} \quad (6)$$

$$m_b = m_{star} - 2.5\log(\frac{F_b}{F_{star}}) \quad (7)$$

where F_b is the flux of the companion obtained from aperture photometry, F_a is the flux of the peak in the centre of the mask obtained from aperture photometry, T is the transmission of fluxes, F_{star} is the flux of the star calibrated with transmission, m_{star} is the apparent magnitude of HIP16095 and m_b is the apparent magnitude of the possible companion. Apparent magnitude defined as the brightness of a celestial object as it is actually measured from the earth. Absolute magnitude defined as the brightness of a celestial object as it would be seen at a standard distance of 10 parsecs (pc). 1 pc is an angular unit of distance which is equivalent to 3.26 Light Years.

Preliminary Results

Candidates are found from our data set. A possible companion of HIP 16095 has been selected to discuss in this report. HIP16095 is A0V type star with 6.311 magnitude (mag) in the K band, which is located 103.66 ± 1.38 pc away (Collaboration (2016)). More K′, L and H band observations have been processing at the moment.

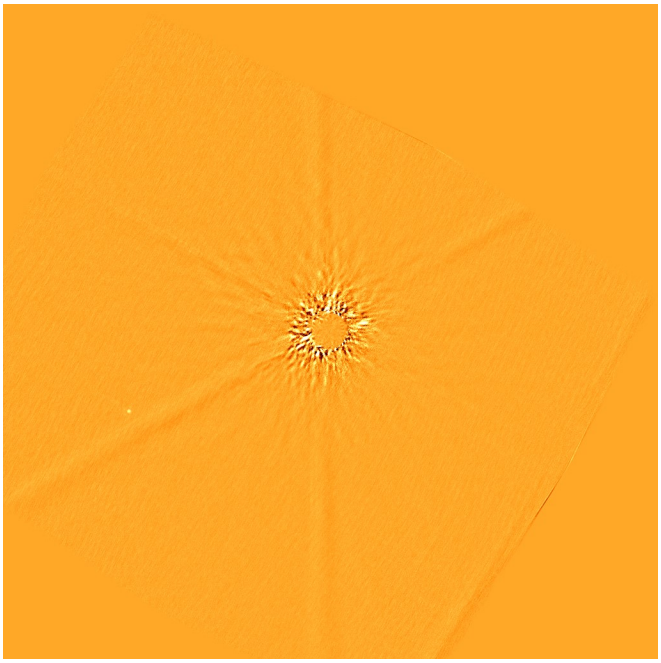


Figure 4: HIP16095 and the potential candidate after ADI reduction in the K′ band from 2013 October observations.

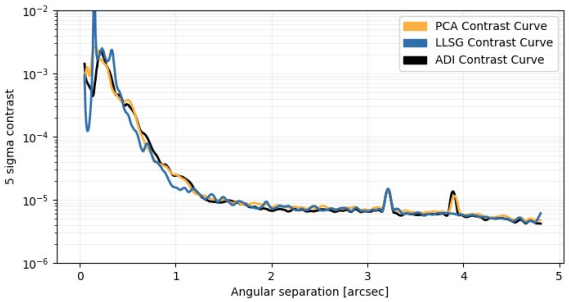


Figure 5. Contrast curve of HIP16095. The peak at ≈ 3 arcsec indicates an increase in contrast, in this case, the position of this peak matches the location of our potential candidate in the final processed image. The second peak is likely caused by the residual starlight, which corresponds to the bright fringe occurred at the lower right corner in figure 4.

Figure 4 shows the presence of the potential candidate after ADI reduction in the K′ band from 2013 October observations. This candidate is located ≈ 3.3 arc second (≈ 340 AU) away from its host. Three algorithms have confirmed the presence of this candidate. In addition, other observations which spaced more than 6 months apart have also identified this companion.

Figure 5 demonstrates the 5-sigma sensitivity limit which shows the detectable contrast for the point-like sources as a function of the angular separation from the star. The first peak corresponds to the potential companion. The second peak is likely caused by residual starlight caused by the geometry of the primary mirror. Both PCA or ADI were not able to remove the speckle pattern completely. LLSG has demonstrated its capability of removing residual starlight from the final reduced image. In addition, the contrast curve has shown its dependence on the post-processing algorithms and their tuning. To evaluate the performance of each algorithm, ROC analysis is required. A receiver operating characteristic (ROC) analysis is required if we would like to quantify the performance of each algorithm. ROC curve is the comparison of two operating characteristics (True positive rate against False positive rate) as the criterion varies. Table 1 presents the results of the aperture photometry in the H and K′ band and (H-K) indicates the colour of the possible companion. From the table, it is clear that scatter exists in the (H-K) colour space. This may due to the nature of our method for deriving fluxes of the companion because the scatter is originated from the K′ band magnitude. Therefore, direct measurement of fluxes behind the coronagraph may be a better way for a more precise photometric result.

Due to the time limitation of the summer internship, further investigations cannot be finished within the time frame. More analysis and tests are required to characterise this possible companion. A detailed proper motion analysis has to be carried out to confirm if the potential companion is locally bounded by the host star.

Proper motion is the part of the apparent motion of a fixed star that is due to its actual movement in space relative to the Sun. Furthermore, spectroscopic study and models should be carried out so the physical properties of this candidate can be estimated.

Conclusion

High contrast imaging offers a window for direct detection of the planetary system. Processes like planet-disk interaction and planet formation can be observed and studied in detail. The discovery of candidates in this survey requires further investigation for validation and characterisation. For instance, detailed proper motion analysis, precise photometry and direct measurement of the candidate′s spectrum.

Filters	Epoch	Coadd	Integration time per coadd(s)	m	M	(H-K)
H (600)	2017nov07	2	6	≈17.5	≈12.42	null
Kp (600)	2016nov11	2	4	≈17.24	≈12.17	≈0.25
Kp (600)	2015jan 10	4	3	≈17.15	≈12.07	≈0.35
Kp (600)	2014oct 03	10	3	≈17.66	≈12.58	≈-0.16

Table 1. Information of HIP16095 in H and K′ band from our observations. Photometry is performed for estimating the colour of the companion. m and M are the apparent and absolute magnitude respectively.

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UgSJ Undergraduate Science Journal University of Exeter

Could new evidence and the new UK government strategy support a phage therapy trial on the Peninsula?

Neil Riley

Introduction

It took a lot for science to inspire '80s rock. Back then, the popular imagination was sufficiently seized by the fear of nuclear apocalypse for Iron Maiden to directly quote the Bulletin of Atomic Scientists. The world was stood at "two minutes to midnight, the hands that beckon doom." According to the current UK Chief Medical Officer in her foreword to the recent government strategy to address anti-microbial resistance (AMR), we face a different 'apocalypse' (Global and Public Health Group, 2013). In 2015 the same government group predicted 10 million global deaths from AMR infections by 2050, up from 700,000 in 2013. This level of loss is similar to the nuclear 'midnight' that paralysed the world in the fear of the Cold War years. In contrast, there is far more the hands of the healthcare community can do to avert an antibiotic apocalypse, including measures that we could have taken a century ago. 2017 marked the 100th anniversary since the first clinical trial treating cholera using the natural viral enemy of bacteria – bacteriophages (d'Herelle, 1917). By the standards of the time, it was successful, spawning the Georgian Eliava Institute still treating patients today (Wittebole, De Roock and Opal, 2014). However, overtaken by the easily administered wonder drugs flowing from Fleming's findings, phage therapy was stuck as a curious cottage industry on the wrong side of the Iron Curtain.

Basic research into bacteriophages, commonly 'phages', has advanced since 2013. Professor Martha Clokie has been building the knowledge base for over a decade, a long way from the sea in Leicester. Having started her career with oceanic bacteria, she is a strong advocate for a resurgence for phage therapy. In her terms, bacteriophages prosper anywhere there are bacteria in sufficient numbers (Clokie, 2018). Previous Exeter research into *E. coli* and associated antimicrobial resistance genes (ARGs) in the waters surrounding the Peninsula sets the conditions perfectly for a wave of bacteriophage research (Leonard *et al.*, 2018). This could in turn support future translational studies in the universities and healthcare establishments clustered around our southwestern shores.

Bugs for breakfast

Bacteriophages have two modes of interaction with host bacteria, lysogenic and lytic. In the lytic mode, the phage kills the host as soon as it has replicated sufficiently, rather than living a co-existence which may co-evolve towards resistance. Fig 1 provides a basic schematic of a phage. attacking a bacterial cell, it's DNA stabbing into the cell like a knife into butter. The more refined 'obligately lytic' is defined as "upon infection is inherently unable to display lysogenic cycles or chronic release" (Bryan et al., 2016). The T4 class of bacteriophages in which the most viable candidates belong is obligately lytic (Villarroel et al., 2017). Although the classic translation of phage may be to 'eat' bacteria, it is more accurate that phages 'break' bacteria, and fast.

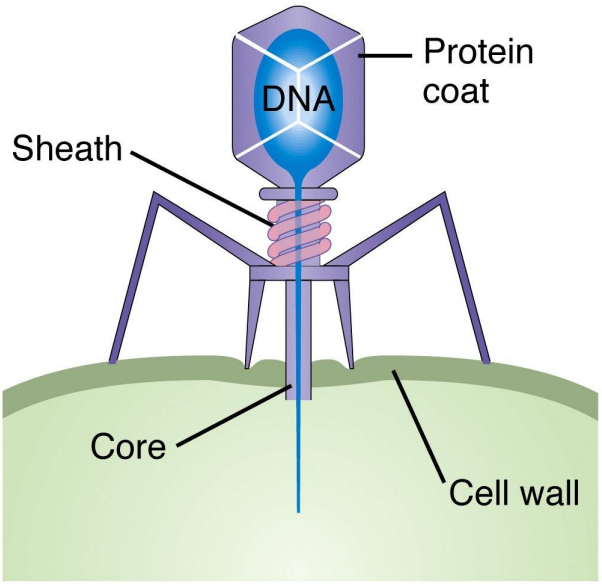

Fig. 1The basic structure of a bacteriophage. Infecting a bacterial cell. Credit Dr Steven Carr. University of Newfoundland (2015)

Targeting Sepsis

There are 250,000 cases of sepsis in the UK each year, 20% of which spread to the system from a urinary tract infection (UTI) (*THE SEPSIS MANUAL*, 2017). *Escherichia coli* was found present in 41% of patients in a recent Eliava trial applying bacteriophages after surgery to the urinary tract. The September 2016 government response to the AMR Review set a specific target of 50% reduction in *E. Coli* infections (DoH, 2016). Basic science, experimental and trial progress in recent years justifies greater prominence of phage therapy as an active solution to counter several classes of AMR bacterial infection. There is a case for the establishment of a randomised clinical trial to compare the use of lytic bacteriophages in *E. Coli* UTI management, in comparison to the established antibiotic protocol. An increasing proportion of *E. coli* infections involve extended-spectrum beta-lactamase (ESBL) strains, which are resistant to all but a small number of conventional antibiotics. Hence, even if a trial population suffers a relatively low incidence of AMR infection, achieving sufficient power in an RCT would beckon swift introduction of a smart alternative to antibiotics.

In addition, the specificity of phage therapy to exclusively pathogenic bacteria would offer superior overall patient outcomes and a dramatic reduction of the side-effects inherent in antibiotic treatments.

High calibre trials and a more subtle knife

Evidence from non-intravenous trials is not strong. By a recent niche internet cartoon, "phages may kill bacteria in a petri dish, but so does a hand gun, and we wouldn't use that in a patient." The author has expanded this analogy in fig 1. We have continued to blast away at bacterial infections in the great petri dish of the global population. With each shot, we've given the bacteria another fragment of our conventional weaponry, presenting a selective pressure that has driven their evolution strengthening ARGs. Meanwhile, we've struggled to lift the subtler knife of bacteriophages from a firm lodging in the research bench. While perhaps unwisely stoking public concern over virus use in general, this squarely hits the problem of reproducibility from the strong theoretical basis. Phagoburn was a European collaboration, multi-centre RCT into the efficacy of phage-soaked dressings to suppress infections in burns patients. Critically, perhaps, it lacked a placebo arm. Hence, while conventional treatments significantly out-performed the phages, the phages were not rigorously compared to placebo. If we are to reduce conventional use, this may be enough, but better control would have proven this and allowed quantification. Similarly disappointing results have come from orally administered trials in Bangladesh and the US (Sarker and Brüssow, 2016). As an astute blogger on the Phagoburn results noted, it may be that these means of delivery fundamentally fail to deliver sufficient numbers of phages to the target bacteria. Hence, any mechanism that puts the phages in more consistent contact with their target bacteria is likely to be more effective, allowing them to amplify through cycles of replication and lytic action. Indeed, the proliferation of phage therapy through the blood may be the only way for it to reach its potential strength in combatting the biofilms that make E. coli and the like quite so hard to kill (Laverty, Gorman and Gilmore, 2014).

Experimental Success

Tom Patterson is a UC San Diego lecturer saved in 2016 from an otherwise lethal, highly resistant *Acinetobacter baumannii* infection by experimental intravenous phage therapy. *A. baumannii* is a similar but rarer Gram-negative rod than *E coli*. The case is eloquently presented by Steffanie Strathdee's TEDx lecture 'Sewage Saved My Husband's Life', and well documented by the clinician who led the experimental treatment (Schooley *et al.*, 2017). In light of their experience, this group founded the Center for Innovative Phage Applications and Therapeutics (CIPAT) in 2017, closely allied to at least

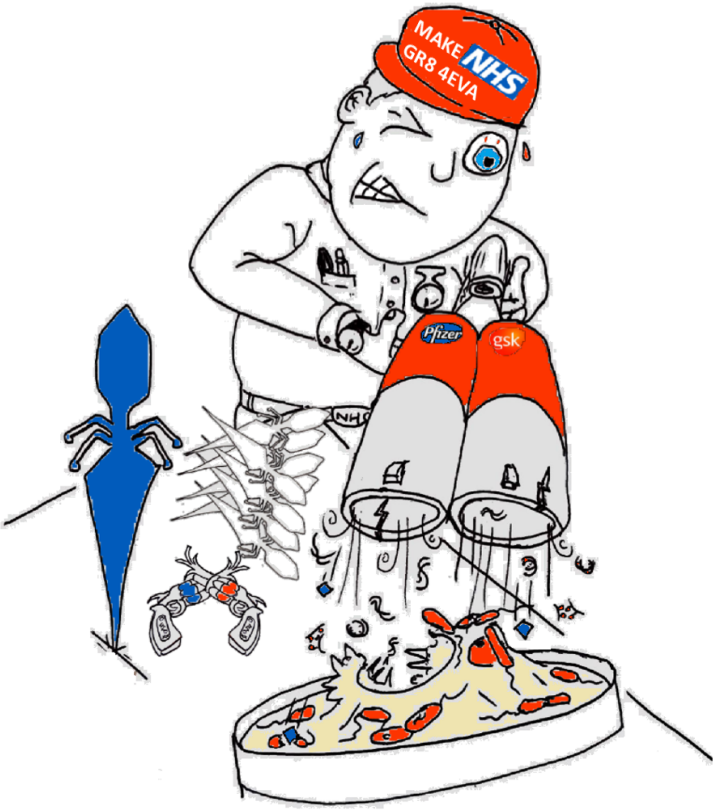

Figure 2. Conventional antimicrobial shotguns or a more subtle knife?

one commercial biosciences enterprise, AmpliPhi. The firm has submitted applications for formal trials using the same IV approach which saved Patterson, replicated in subsequent cases. It may be that a putative Peninsula trial, Pen2Phage until a better name emerges, would follow a lead from San Diego. However, regulatory constraints may be more efficiently adapted here, and a greater scale achieved within the power of the dynamic institution the NHS truly is.

Surveillance and Potential Trial Population

The October 2018 report of the English Surveillance Programme for Antibiotic Utilisation and Resistance (ESPAUR) provides useful data to inform the prioritisation of specific infections. Estimating the total number of blood stream infections across England at 83,079 cases, of which 16,504 were resistant to antibiotics, the report comments that *E. coli* was responsible for 41,287 in all, of which 12,030 were resistant (Eurosurveillance editorial team, 2012). Further, 439 cases were resistant to colistin, our current antibiotic of last resort. *E. coli* accounts for 50% of all blood stream infections, and 73% of AMR infections. Fig 3 is a coloured electron micrograph demonstrating the potential efficacy of phages in stabbing into *E. coli*. If a putative Pen2Phage RCT recruited a neat 5000 to all arms from the population in these numbers, it would be a well powered trial, even if limited to the Peninsula.

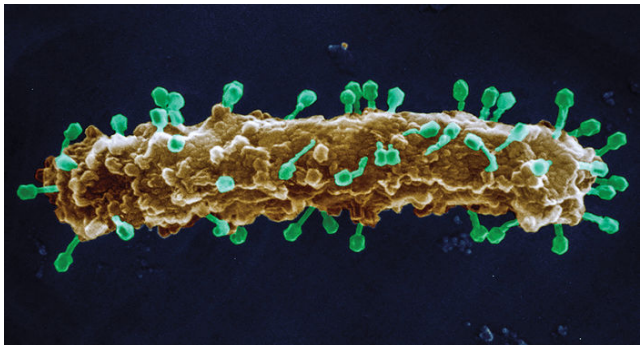

Figure 3. T4 Phages studding an *Escherichia coli* bacterium. EYE OF SCIENCE/SCIENCE SOURCE.

Soil, Sewage and Sea

By Strathdee and Clokie, phages are all around us, feasting on our microbiome and flowing with our waste into the sea. The search for conventional antimicrobials has moved from the soil to the water. Where we find bacteria, we find phages (Clokie, 2018). It may be that fishing for phages in the same places, including the sewer and the surf, is both practical and necessary. Effective phage therapy would rely on access to a plethora of potentially suitable phage strains to allow adaptation of those used, perhaps 'selective breeding'. This capability may be key to the Eliava Institute's success, given their broad repository of phage strains and experience in managing them (Wittebole, De Roock and Opal, 2014). In developing a phage therapy suited to E. coli, digitised sequencing for both target bacteria strain and corresponding treatment phage strains may make their use similarly swift to antibiotics, but with dramatically increased precision. The refinements required in frontline diagnosis may be coming anyway, with increased capacities to sample and sequence infection to identify strain, rather than just species, at suitable pace and price.

Viral Safety and Public Perception

It is well accepted that T4 phages occupy a tightly constrained niche in their infection and lysis of bacteria (Clokie et al., 2011) and are highly unlikely to adapt in vivo to infect human cells instead (Bryan et al., 2016). Indeed, the far more emotive human immunodeficiency virus may increasingly be used in developing human therapy (Connolly, 2002). On balance, it seems a fair assessment that Lentiviruses carry more inherent risk than bacteriophages. Natural, non-engineered T4 phages which could be administered intravenously against blood stream infections present no significant threat to human cells. Large scale RCTs must prove this conclusively, but the experimental cases are supportive. Realisation of the UK AMR strategy educational strand should cast phages as allies in a battle against bacteria and hence support their swift acceptance as clinically useful agents for human good.

Conclusion

Healthcare professionals and allied researchers have worked hard in recent years to comprehend antimicrobial doom, and now need the full support of society and government to vanquish it. There was sufficient evidence to support the recent surge in trial activity, building despite relatively underpowered, weak results. Little has happened in the UK, and nothing on our Peninsula. Given the success of intravenous administration in severe infection experimental settings, and the potential payoff in both countering AMR and reducing sepsis risk, it seems wholly reasonable to include phage therapy within a trial design. Even the revised UK AMR plan does little to immediately enable this. The weak trials of other application routes seem to leave intravenous injection as the most viable route, but this must be proven in progressively larger trials. Societal concerns over use of viruses in humans must be addressed by refined focus in education campaigns to champion new solutions, rather than merely admiring the problem. If we're going to wind back the Doomsday clock, it is time for any respectable UK research university to put Pen2Phage on a trial.

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UgSJ Undergraduate Science Journal University of Exeter

What prevention strategies in the UK, in relation to screening and interventions, are required to tackle foetal alcohol spectrum disorder (FASD)?

Sizar Doski

Abstract

Use of alcohol during pregnancy is a significant public health issue in the UK. Foetal alcohol spectrum disorder (FASD) is the number one intellectual disability in the developed world. It is completely preventable through the elimination of drinking whilst pregnant. Significant studies have been undertaken in the US and Canada in comparison to the UK. The aim of this review is to highlight key prevention strategies for FASD, with focus on a prevention model produced by Canada, screening of alcohol consumption, alcohol brief interventions (ABIs) and targeted preventions for 'high-risk' women. Data was collected through keyword searches in research databases and no specific date exclusions were made. The Canada approach for FASD prevention is effective and should be implemented in the UK. The use of screening questionnaires is helpful in assessing risky behaviour, but further research should investigate an effective screening method for assessing maternal alcohol consumption. ABIs should be a key part of antenatal screening delivered by appropriately trained healthcare professionals. Children with lower socioeconomic status and unplanned pregnancies should be targeted for future prevention of FASD. Further research should also assess the effectiveness of ABIs in pregnant women

Introduction

Use of alcohol during pregnancy is a significant public health issue in the UK (Bell et al., 2016). Foetal alcohol spectrum disorder (FASD) is described as the number one intellectual disability in the developed world (British Medical Association, 2016). It is completely preventable through the elimination of drinking alcohol during pregnancy. Significant research in Canada and the US has focused on FASD and prenatal alcohol exposure (PAE) in contrast to the limited reports in the UK (McQuire et al., 2019).

A collective responsibility with clear and consistent advice from policy makers, targeted measures that support women without social stigmatisation or apportioning blame is key for preventing FASD (British Medical Association, 2016). This review will highlight some key prevention strategies. It will focus on a prevention model produced by Canada, the use of screening questionnaires, alcohol brief interventions (ABIs) and targeted interventions for 'high-risk' women as a method to tackle FASD.

Methods

A literature search using the University of Exeter Electronic Library was conducted with the search terms: 'foetal (or fetal)', 'alcohol spectrum disorder' or 'FASD', 'prevention'. The same criteria were applied to Scopus, Cochrane and Tripdatabase. Research articles were critically appraised using the Critical Appraisal Skills Programme (CASP) toolkit.

Results and Discussion

Damage from prenatal exposure to alcohol is dependent on the level and pattern of alcohol consumption, the environment, genetics and also the stage of pregnancy that alcohol was consumed (Boyle, 2013). The foetus is unprotected from circulating maternal alcohol which can impact the central nervous system and foetal brain development (Mukherjee, 2006). This damage can be accompanied by a variety of cognitive and behavioural problems, physical and emotional developmental problems, and commonly, but not always, a set of distinctive facial dysmorphology (McQuire et al., 2019). In addition, more than 400 adverse outcomes are associated in affected individuals, including mental illness and substance abuse (Streissguth et al., 2004). FASD is a non-diagnostic umbrella term used to describe a set of disorders caused by PAE - other definitions are summarised in Table 1.

Table 1. Foetal alcohol spectrum disorder (FASD) is an umbrella term to descibe the main subtypes and core features summarised. Adapted from McQuire et al (2019).

	Foetal Alcohol Syndrome (FAS)	Partial Foetal Alcohol Syndrome(pFAS)	Alcohol-related neurodevelopmental disorder (ARND)
Prenatal alcohol exposure	May be unconfirmed	Confirmed through screening	Confirmed through screening
Impairment of 3 or more subdomains of the central nervous system	Yes	Yes	Yes
Facial anomalies	Three features	Two features	Not required
Growth deficiency	Yes	Not required	Not required

Data from USA and Europe suggest that FASD can affect up to 1% to 10% of the population (Lange et al., 2017). A new study by McQuire et al. (2019) demonstrates a prevalence of 17% in the UK which is significantly higher than the estimates of Europe and the US, but in the upper limits of worldwide prevalence. In addition, PAE in the UK is significantly higher than the US, with pooled prevalence of 41% compared to 15%, respectively. Consistent with this study, the Office for National Statistics (2018) highlighted that 57% of respondents in the Opinions and Lifestyle Survey aged 16 years and over in 2017 drank alcohol. It was also reported that 25.6% of women aged between 16-24 binged alcohol. The Chief Medical Officer in 2016 stated that the safest approach for pregnant women or those that may become pregnant is to abstain from drinking alcohol (Department of Health, 2016). Drinking alcohol is accepted as a normal part of daily living in the UK (BMA, 2016). Therefore, the consumption of alcohol during pregnancy should be explored in the context of society's relationship with alcohol – not in isolation.

Role of healthcare professionals

Healthcare professionals are ideally placed to implement prevention strategies. Women are likely to benefit from different types of support and interventions since there are many potential reasons that mothers may consume alcohol during pregnancy. This may include consumption of alcohol before the pregnancy is recognised, lack of awareness of the risks, social pressures and alcohol dependence (Jonsson et al., 2014).

A multi-faceted and coordinated approach is required to tackle motivations that result in alcohol consumption during pregnancy. In the past decade, a holistic approach to prevention and management for FASD has been developed in Canada (Public Health Agency of Canada, 2008). The approach builds on education that raises awareness of FASD and focusses on improving the woman's health such as nutrition, stress reduction and a focus to find the root of alcohol or addiction problems (BMA, 2016). A summary of this approach is found in Table 2.

Table 2. Adapted from the Public Health Agency of Canada (2008) and the British Medical Association (2016). A summary of the foetal alcohol disorder spectrum (FASD) prevention approach in Canada.

Level 1	**Health promotion and FASD awareness** Elevating public awareness of FASD – non-specific approach.
Level 2	**Discussion of alcohol use and risks** System wide approach from different healthcare professionals to all women of childbearing age and their support network to discuss: • Use of alcohol and risks • Other substance use • Coping without alcohol • Available prenatal support • Pregnancy planning
Level 3	**Drinking pregnant women with health and social problems** Specialised support for pregnant women. The approach is holistic, non-judgemental and culturally relevant. Help with nutritional status, housing, ability to retain custody of their children and treatment for substance use (Motz et al., 2006).
Level 4	**Postpartum support** Maintain or initiate health and social problems. Will also provide support for the children's development. May also include early intervention for the child.

A significant aspect of this approach is aimed at the wider support network of child-bearing women, such as close family and partners. This recognises a collective responsibility through a supportive environment to eliminate drinking through pregnancy and consequently reducing the incidence of FASD. Improvements with nutritional status, housing, ability to retain custody of their children and treatment for substance use has been demonstrated by this approach (Motz et al., 2006).

Screening questionnaires

Effective prevention of the range of FASD requires provisions beyond health advice. Accurate monitoring and detection of maternal alcohol consumption and the implementation of evidence-based interventions is important for effective reduction of PAE (BMA, 2016). The current National Institute for Health and Care Excellence (NICE, 2014) guidance on antenatal care includes the provision of advice rather than screening of alcohol use during pregnancy. The Scottish Intercollegiate Guidelines Network (SIGN, 2019) guideline has recently been published to provide clear guidance on monitoring and recording of alcohol consumption as part of routine screening in antenatal care.

Screening questionnaires may facilitate the identification of alcohol consumption during pregnancy and risky drinking behaviours. The BMA (2016) has highlighted the importance of these questionnaires as part of antenatal screening, and appropriately trained healthcare professionals to provide this care. A systematic review of five studies (6724 participants) by Burns et al. (2010) found that for risky drinking, a high sensitivity was found for T-ACE (69-88%), TWEAK (71-91%) and AUDIT-C (95%) with high specificity at 71-89%, 73-83% and 85%, respectively (the questionnaires are summarised in Table 3). AUDIT-C in particular, has been highlighted as an effective screening tool that is also useful for identifying alcohol dependency and abuse. All three screening tools has been recommended by SIGN (2019) for detecting the misuse of alcohol among pregnant women.

In contrast to these guidelines, the systematic review by McQuire et al. (2016) was the first to investigate screening questionnaires to identify alcohol consumption in pregnancy. The authors concluded that although the questionnaires could be helpful for assessing risky drinking, biomarkers in the placenta and the meconium could be possible for investigating PAE. However, the dangers of PAE may be raised by the screening process itself – serving as a method of awareness. Thus, implementation provisions should be provided to healthcare professionals to allow screening time for alcohol consumption during pregnancy to be part of routine antenatal screening.

Table 3. Adapted from the British Medical Association (2016). Each questionnaire has a criterion for referral.

T-ACE	Tolerance: 'How much does it take until you feel the effects?' Annoyance: 'Are you annoyed by criticism of drinking habits?' Cut down: 'Have you ever thought about cutting down?' Eye-opener: 'Have you ever had a drink to get over a hangover or steady your nerves in the morning?'
TWEAK	Tolerance: 'How much does it take until you feel the effects / what is the most you have had in one sitting?' Worried: 'Have your family or friends ever been worried about your drinking habits in the past year?' Eye-opener: 'Have you ever had a drink to get over a hangover or steady your nerves in the morning?' Amnesia: 'Do you ever not remember what you did the night before after drinking?' Cut down: 'Do you feel the need to cut down on your drinking?'
AUDIT-C	'How often do you consume alcohol containing drinks?' 'On a typical day of drinking, how many drinks containing alcohol do you have?' 'How often do you have six or more drinks?'

Brief interventions

Brief interventions are required before or soon after identification of alcohol-related problems as a prophylaxis for further damage. It is grounded on understanding, predicting and changing human behaviour based on the social cognitive theory. Alcohol brief interventions (ABIs) consider both personal and contextual factors for drinking. Important component of ABIs draws on the mother's self-efficacy about changing their drink, beliefs and attitudes towards drinking and a normative comparison with other people's drinking habits (Boyle, 2013). Brief interventions are delivered depending on the stage of pregnancy, severity of alcohol dependence and the level of risky drinking behaviours (summarised in Table 4).

Healthcare professionals require the time and resources to ensure identification for women that are pregnant, or planning a pregnancy, with a suspected or confirmed history of alcohol consumption, to be offered an ABI at the earliest possible stage. ABI is a cost-effective strategy underlined by the SIGN (2019) clinical guidelines in response to The Scottish Government (2013) priority for implementing ABI in antenatal care. It has been found to produce clinically significant effects on drinking behaviours, particularly in women that consume low-to-moderate amounts of alcohol and are non-dependent (NTASM, 2006). Robust evidence from over 40 systematic reviews have been published, ranging from primary care, emergency care, hospital, social care and also in community settings (Boyle, 2013). The reported reduction of risky drinking (Moyer et al., 2002), particularly on its reduction of drinking quantity, intensity and frequency (Kaner et al., 2007) has been reported in an extensive range of settings for non-treatment seeking populations (Kaner et al., 2011). However, there is limited research on ABI for women that are pregnant based on a systematic review in primary healthcare by O'Donnell et al. (2014).

The need for ABI is supported by comments from a mother of a child with FASD "*I asked whether I could drink wine during my pregnancy, he [the physician] did not ask whether I had a drinking problem, or how many drinks I had a day… I was angry because I was given the wrong information*" (Alcohol Free Pregnant, 2012). It is reported that delivering an ABI requires around 25 minutes in the UK, which is unsustainable in many healthcare settings. In contrast, brief interventions in Sweden were successfully delivered in less than 5 minutes (Kaner et al., 2007; McCambridge and Saitz, 2017). However, it remains that routine screening of alcohol during antenatal care provides an important opportunity to deliver an intervention to reduce alcohol consumption. This should be delivered by trained healthcare professionals (NICE, 2015).

Table 4. Summary adapted from the NHS Health Scotland (2015) advice for alcohol brief interventions (ABIs). ABIs should be based on the FRAMES structure: feedback, responsibility, advice, menu, empathy and self-efficacy (Boyle, 2013). Brief interventions can be extended so that a series of three to twelve structured interviews are delivered by a competent practitioner (NICE, 2010).

Stage 1	**Raising the issue** Identifying the amount of alcohol consumed.
Stage 2	**Screen and give feedback** May use the AUDIT-C, TWEAK or T-ACE to identify drinking habits in comparison to national guidelines.
Stage 3	**Listen for readiness to change** Asking the patient how they feel about what was discussed.
Stage 4	**Suitable approaches** 1) Information and advice 2) Enhance motivation 3) Menu of options 4) Building confidence 5) Coping strategies.

Targeted prevention

Encouraging evidence for strategies that target high-risk or pregnant women has emerged (NHS Scotland, 2015). Healthcare professionals have a responsibility to implement targeted interventions when women have been identified as high-risk of PAE. The SIGN (2019) guidelines has recommended further research on targeting high-risk women, which means the UK still has no specific guidance on the delivery of targeted prevention. Whereas, several innovative strategies that are currently being trialled in the US. 'Protecting the next pregnancy' is a project in the US

that targets women with alcohol exposure in previous pregnancy which is followed by intensive ABI. Improved birth outcomes were reported with reduced alcohol consumption (BMA, 2016).

The NTASM (2006) has stated that individuals with severe alcohol problems, including dependence, should be referred to a specialist alcohol service due to a lack of evidence for ABI. Considerations should be made for pregnant women or those trying to conceive. A study by McQuire et al. (2019) has found a high FASD screen among children with lower socioeconomic status and unplanned pregnancies, which should also be a target for healthcare professionals. The approaches include relapse prevention, detoxification facilities, inpatient residential programmes and outpatient clinics. Co-ordinated multi-agency plans are recommended for substance misusers in pregnancy by NICE (2010b). This encourages midwives and doctors to refer alcohol misusers to additional services when identified.

Conclusion

To tackle the preventable and completely irreversible condition of FASD, it is necessary to consider adopting the Canada model for prevention, screening patients effectively for PAE, the use of ABIs and targeted prevention for high-risk mothers. Healthcare professionals require the time and resources to ensure ABIs are undertaken at the earliest possible stage. Further research to assess the effectiveness of ABI in pregnant women should be undertaken. Further research should also examine the most effective screening method for assessing maternal alcohol consumption.

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UgSJ Undergraduate Science Journal University of Exeter

How effective are climate models at representing European wind storms?

Naomi P. Faulkner

Abstract

This project uses statistical modelling to analyse how high-resolution storm footprints from the Windstorm Information Service (WISC) compare with observation data from weather stations around Europe. A model is used to make predictions of these windstorms, namely predicting the highest wind speed at the observation locations and a lower resolution footprint, particularly focussing on the storm Jeanette (Oct 2002). Then the effectiveness of the WISC storm footprints in representing the characteristics of a windstorm is examined through interpreting the results from the model and the predictions produced. It was found that whilst the WISC footprints are accurate in representing the wind speeds in the vicinity of the storm track, where wind speeds are highest, the footprint wind speeds away from the storm track generally tend to be lower than those of the statistical model. Further investigation is needed to ascertain which model's speeds are closest to actual wind speeds.

Introduction

European windstorms are extratropical cyclones that track across the North Atlantic Ocean towards western Europe. Usually, their path is directed over the Norwegian Sea, but when they travel further south, they have potential to cause widespread damage in European countries. For example, January 2018 saw windstorms Friederike, Eleanor and Carmen hit areas in western Europe, which had many devastating impacts, including a $2+ billion loss for the insurance and reinsurance industry (Artemis, 2018). Since there is an average of 4.6 windstorm events per month from November to April (Renggli, 2011), it is important to further the knowledge surrounding these events – their pattern, characteristics and predictability – in order to increase the accuracy of assessing risks associated with extreme windstorms and hence reduce/prevent these losses.

The Windstorm Information Service (WISC) is part of the Copernicus Climate Change Service (C3S) and collaborates with insurance sectors to improve knowledge around these events by producing high-resolution footprints of European windstorms, amongst other windstorm analyses. However, it is generally understood that these footprints do not always best represent storm events. Some footprints appear to be relatively accurate in the way they characterise a storm when they are compared to observations and scatterometer readings (WISC, 2016), whilst other footprints do not seem to be as reliable.

A similar statistical problem has been investigated on the topic of air pollution: a study looks at improving the model to be more aligned with the observation data (Fuentes & Raftery, 2005). The statistical problem is also strongly related to post-processing of, for instance, weather forecasts. A model produces a weather forecast, systematic discrepancies between forecasts and observations are quantified, and then the discrepancies are addressed to correct the forecast. For example, a study carried out by Raftery et al. used Bayesian model averaging for postprocessing ensembles (Raftery et al., 2005).

This report explains how statistical models were used to investigate if and where WISC storm footprints fall in accuracy in terms of representing a storm.

Methodology

Data

WISC has produced what are called historical storm footprints by collating the data from ERA-Interim, a global atmospheric reanalysis from 1979, and ERA-20C, an atmospheric reanalysis from 1900 to 2010 using observed surface pressure and surface marine winds (ECMWF, 2018).

The WISC footprint dataset contains 148 storms in the period November 1940 to December 2013, which are considered the most severe storms in this timeframe (WISC, 2018). WISC uses a rotated pole to produce these footprints, so their plots have a non-rectangular domain when standard coordinates are used. Each footprint has a 1637 x 928 resolution, with each grid point corresponding with that location's maximum wind gust (m/s) in a 1637 x 928 matrix.

The NCDC GSOD (National Climatic Data Centre Global Surface Summary of the Day) database contains observation data of these storms from weather stations located around Europe. This database comprises of the maximum wind gust speeds measured at stations within the timeframe that the footprints are defined. Each storm is defined by 72-hour periods, so the data are the maximum of the three days' maximum gusts for each station. The number of observations for each storm noticeably differs, with some storms having few station observations and some having over a thousand, especially since data become increasingly scarce in the years earlier than 1979. Therefore, only the latter 58 storms since 1979 in the dataset were used in this project, in which there are on average 724.12 total observations for each storm.

The elevations of each location in the footprint and each weather station location is determined by interpolating a data frame containing the Earth's surface elevation above sea level onto the relevant grid points. Each of the observation data points were assigned a categorical factor, with levels 91

to 148, to represent which storm in the dataset the observations corresponded to. These multiple data sets were assembled into a single data frame for the modelling process

Model

The model was fitted in the programme R with the station observations as the dependent variable: a mixed effects model was used so that it would consider how each storm's characteristics can be random. So, whilst some variables that are determined for a storm are fixed e.g. longitude (Lon), latitude (Lat), etc. There is also a random component in the way a storm develops.

The final model was selected by balancing the simplicity of the model and its effectiveness:

$$\text{observation} \approx \text{f(s)} + \text{elev} + \text{footprint} + \text{g(s):footprint} + \text{h(s):elev} + \text{elev:footprint} + (1+\text{footprint}+\text{g(s):footprint}+\text{elev:footprint}|\text{storm})$$

Where,

$$\text{f(s)} = \text{Lon} + \text{Lat} + \text{Lon}^2 + \text{Lat}^2 + \text{Lon:Lat}$$

For instance, 'g(s):footprint' represents the interaction terms between the footprint value and the spatial variance. The variable 'storm' is included as a random effect to account for the randomness of the intensity of wind gust speeds in different storms and by including the 'footprint' variable (and all its interactions) in the random effects term, the model takes into account the variability in how reliable the WISC footprint readings are from storm to storm.

Results

Observation Predictions

The model is able to predict the observations for each storm by inputting the locations of each weather station that gave an observation and the corresponding elevation and footprint values. Comparing the predictions for Jeanette with the actual observations reveals that, overall, the predicted observations match the actual observations reasonably well (Figure 1). The correlation coefficient between the footprint values and observed gusts is 0.5657, whilst the correlation between the predicted gusts and observed gusts is 0.6613, suggesting that the model fits the observation data for Jeanette more closely than the WISC footprint. Moreover, the fact that the model generally predicts higher wind speeds than the WISC footprints is supported by the almost 6% increase in the mean of the correlation between predicted and observed gusts across the 58 storms. Thus, there is suggestion that the model corresponds with the observations more accurately than the WISC footprints.

Footprint Predictions

Similar to predicting the weather station observations, the model can predict the footprint of a storm by inputting grid point locations into the model, along with the corresponding elevation and footprint values.

Upon observation of the predicted footprint of the storm Jeanette (Figure 2b), the areas where the storm hits worst (around the storm track) substantially correspond with the original footprint (Figure 2a). This shows that the WISC footprint is reliable when representing the areas where wind speeds are highest. There are two major areas that cause concern in the predicted footprint: the area of high wind speed over the Norwegian Sea and area of "negative" wind speed over the Black Sea which are not depicted in the WISC footprint. Since the majority of weather station observations are land-based, the elevation values for these are all positive. However, the elevations of grid points that represent marine locations are largely negative, meaning that the model extrapolates for sea-based locations, and there is no evidence that suggests this extrapolation is reliable. Hence, this explains why these areas are largely different from the original footprint. Also, there are areas over land which do not correspond, where station observations become more infrequent, especially in Turkey. Therefore, these areas are where the data have been extrapolated spatially and in terms of elevation.

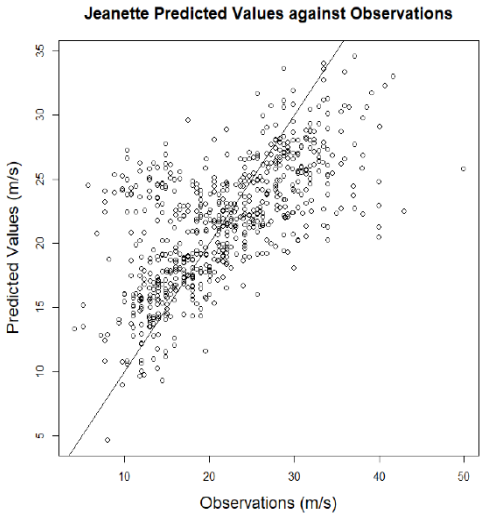

Figure 1: Jeanette predicted gusts against observations, with a correlation coefficient of 0.6613 between the two variables.

By looking at the ratio between the predicted and original footprint for Jeanette (Figure 2c), it is evident there are areas where the two footprints differ. Regions around the storm track, France, Spain, Italy and Turkey are areas where the ratio is more than 1, where the predicted footprint wind speeds are higher than those in the WISC footprint. This can be determined for the majority of the storms upon examination of the mean ratios between the WISC and predicted footprints of all 58 storms (Figure 2d), where the majority of storms have a mean ratio that exceeds 1.0, and with the mode being between 1.1 and 1.2, showing how the

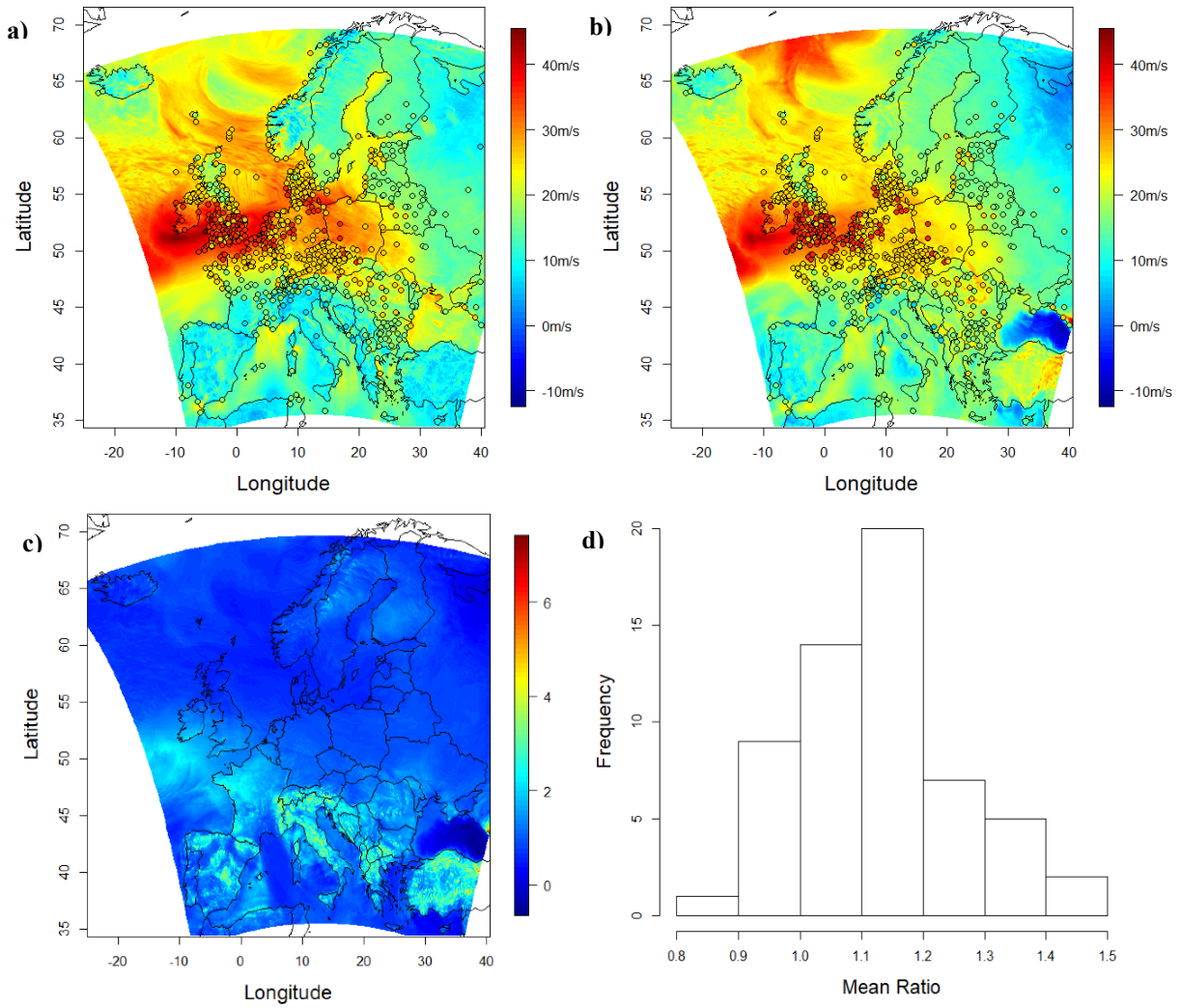

Figure 2: a) WISC footprint overlaid with associated observation data (677 observation points) for storm Jeanette, b) same as a) but for the predicted footprint for Jeanette, c) map of the ratios between the predicted and original storm footprints. Areas where the ratio is more than 1 are where the predicted wind speeds are higher than that of the original footprint, and areas less than 1 are areas where the wind speed is predicted to be lower than the original footprint, d) histogram of the mean ratios between the predicted and original storm footprints.

WISC footprint wind speeds tend to be lower than those of statistical model.

Overall, even though some regions' predicted wind speeds are not reliable (areas over seas and of high elevations), it becomes clear that the WISC footprints are reliable in terms of representing the wind speeds of where the storm hits worst. But it remains unknown whether the WISC footprints or predicted footprints from the statistical model are more reliable at representing wind speeds across the whole domain.

Conclusion

This project has revealed that WISC footprints are reliable when representing the highest wind speeds around the storm track, for example, Ireland, the UK and surrounding seas for the storm Jeanette, where the original footprint largely corresponds with the predicted wind speeds in these areas and the corresponding observations, indicating that these readings are accurate. However, when comparing the original WISC and predicted footprints, the wind speed in the regions such as southern European countries in the case of Jeanette are generally lower than what the statistical model predicts. These are also similar areas to where observations become more infrequent. The fact that the model shows bias in these regions and over marine locations due to unreliable spatial and elevation extrapolation proves how footprint accuracy and reliability can be improved by increasing the spread and number of weather stations. The regions where the model is most biased, the Norwegian Sea and the Black Sea, are where the model has largely extrapolated. Therefore, the model could also be improved by researching how marine elevation affects the wind speeds in European windstorms, to see whether this extrapolation is reliable or not.

This project has primarily concentrated on the storm Jeanette, but the model has the potential to individually analyse the original vs. predicted footprint behaviour for all 58 storms in

the dataset, which may reveal why the reliability of WISC footprints varies from storm to storm.

Acknowledgments I would like to thank Dr B. Youngman and Dr T. Economou for giving me the opportunity to take on this project and teaching me a great deal of new things about statistical models, how to use them and how to apply statistics to real-world problems.

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UgSJ Undergraduate Science Journal University of Exeter

Manipulation of Microplastics in Settling Tanks Using Faraday Waves

Saskia Sherwood

Abstract

An article from The Guardian [G. Wearden, 2016] claims that by the year 2050, there will be more plastic by weight in the oceans than there are fish. This environmental problem has been ignored for most of a century. The aim of this project was to produce a sustainable foundation of reducing the amount of plastic released by water treatment plants. Faraday waves were used in order to manipulate and remove the plastics. At high amplitudes, the electrostatic forces increased, causing faster clumping, and a greater electrostatic attraction of the smaller microplastics, such as microfibers and plastic powders, to the nurdles. Overall, sufficient research was done to design a basic filtration system, which reduced nurdle and microbead movement

Introduction

Microplastics have plagued most waters on the planet for approximately 50 years. The Guardian revealed that just in 2015-2016, more than 40 billion pieces of microplastics were dumped into the oceans [D. Carrington, 2016]. Varying in shapes and sizes, there is no cost-effective method to remove this type of pollution. One sector of microplastics is microbeads, made up of polyethylene, which were once an ingredient in cleansers and toothpaste. Microbeads are now banned in the UK, however, it does not change the fact that there is still a large residue in our waters that affects wildlife. These microbeads are ingested by fish and larvae, rapidly increasing aquatic mortality rates [M. David, E. Bruno, Q. Patrick, S. Armelle, H. Christine, M. Lauriane, M. Olivier, S. Philippe, R. Johan, H. Arnaud, Z. Jose-Luis, 2015 pg 2].

In this investigation, Biology research, specifically Professor Galloway's analysis of marine pollution, and Physics research were combined. Microbeads were tracked when exposed to a standing Faraday wave in order to actuate a movement pattern. Faraday waves are surface oscillations in enclosed bodies of water, when the frequency exceeds its critical threshold. They are crucial for tracking particle movement, which accumulate in different regions of the wave depending on surface tension, and whether the particles are hydrophilic or hydrophobic [G. Falkovich, A. Weinberg, P. Denissenko, S. Lukaschuk 2005 pg 1045].

Knowledge of such movement patterns can prove a starting point to developing more effective techniques for collecting and removing microplastics. This would be applicable to water and sewage treatment plants because they would be removed at the source before being deposited back into the environment.

Preliminary testing

Plastic clumping was investigated to find the best conditions for the fastest rate. A mixture of poly(ethylene-co-ethyl acrylate) and poly(ethylene-co-acrylic acid) were put in a 100x100x2 mm dish on a speaker.

Paired with previous research, the optimal results were found at amplitudes above 1.4V – which was found in prior research to this experiment – to diminish any possibility of meniscus waves. The range that was tested was 1.5 V-2.0 V at 75 Hz. The speaker was turned on and the clumping was recorded. [Fig.1] shows that the clumping rate was at its highest between 1.8 V -2.0 V, as that is where the graph diminishes. This range was carried forwards.

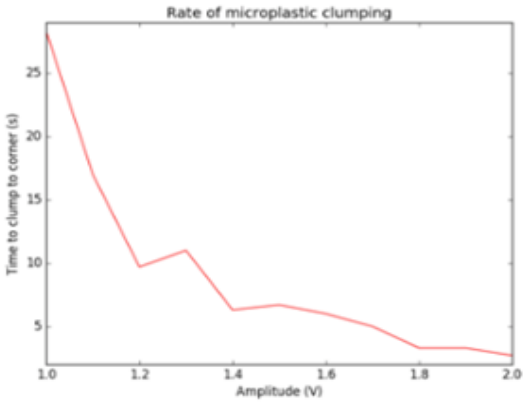


Figure 1: Rate of clumping at 75 Hz between amplitude 1 - 2 V.

Methods

The 150x150x2 mm dish was attached to the speaker and filled to the brim with water. Five drops of silicon oil (20 cSt) and 2.9 g of poly(ethylene-co-ethyl acrylate) nurdles were added. The frequency and amplitude of the speaker was set to 75 Hz, 1.8 V. Images were recorded every 3 - 4 seconds using the Raspberry Pi camera, until the beads were rafted at the corners.

A two-chamber dish [Diagram 1a] was screwed onto the speaker and filled in the same way as the previous experiments. 0.16g of poly(ethylene-co-ethyl acrylate) was placed in each chamber and the amplitude was set to 75 Hz 1.7 V. Note, there was a restricted area on the 3D printer, so the chambers were smaller than the previous dish , hence why less plastic was used. This was repeated for a three-chamber dish [Diagram 1b], and then for a three different sized chamber [Diag. 1c].

[Diag.1c] was then used in the trial filter. The conditions were kept the same as previously, apart from 2 g of poly(ethylene-co-acrylic acid) and 0.5 g of PMA were added. The dish was connected to two tubes at either end, which were connected to a pump. The pump was turned on, to create a water cycle, and so was the speaker at 75 Hz 1.7 V, producing a Faraday wave. No recordings were taken but the experiment was observed in order to confirm if this rough design was optimal or improvements could be made.

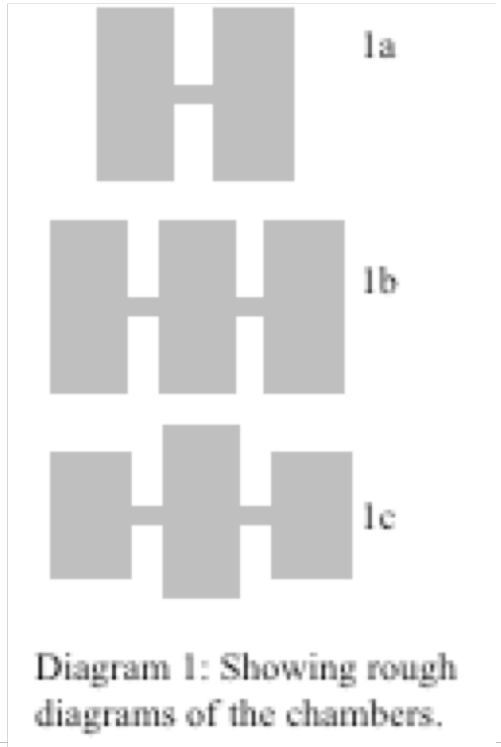


Diagram 1: Showing rough diagrams of the chambers.

Results

The results were graphed using histograms and graphs showing the overall accumulation in an area of the dish (x coordinate is red, y coordinate is green).

The data shows if the beads moved up and down or side to side. [Fig.2] shows that there was a lot of movement in the x and y directions when the amplitude is at 1.8V, regardless of whether the plastics moved in a positive of negative direction. This is due to a uniform Faraday wave, creating enough collisions with enough force causing microplastics raft at the sides of the dish. The results from the experiments at 1.9V and 2.0V were inconclusive and had a limited pattern. This was likely due to the dish becoming lose and disrupting the Faraday signal.

The application of Faraday waves was successful with both poly(ethylene-co-acrylic acid) and poly(ethylene-co-ethyl acrylate). The combination [Fig.2c], shows a great amount of movement in both directions. When observed, they would quickly move to the edges and raft, hence there is a smaller number of frames on the x axis, suggesting that the combination of plastic nurdles and smaller microplastics can be beneficial as the clumping rate is faster, and more direct towards the corners, than when any of the plastics are on their own.

In addition, it is clear when comparing [Fig.2a] and [Fig.2f] that the different plastics do affect each other's movements. From [Fig.2f], and also visual observations, the PMA powder did not move to the corners or clump well. It would fractal and spread, going around in a circle, which was due to the high amplitude causing the particles to move and collide too fast to aggregate. However, the poly(ethylene-co-ethyl acrylate) nurdles, gave the powder more stability as they would stick together causing more powder to move to corners with the nurdles. Hence, [Fig.2a] having a strong negative correlation. As these nurdles are larger than the other microplastics, they have a larger surface area, which as the microplastics are hydrophobic, they would attach onto it and they moved together through the dish [Fig.3]. It was concluded that nurdles were beneficial to the experiment as they increased the clumping rate. They were added to the filter design as they enabled more plastics, and possibly bacteria [FIDRA], to be removed from a filter.

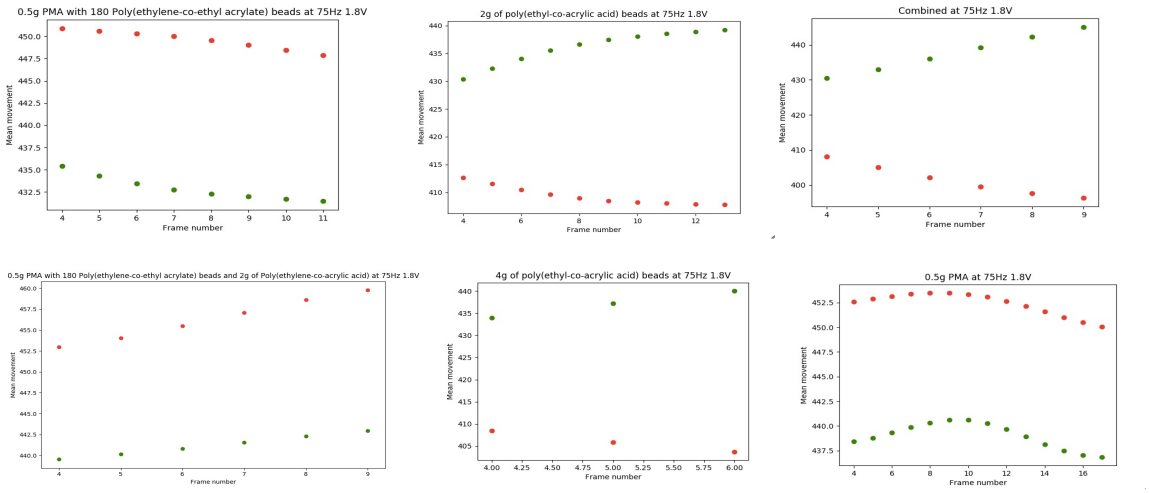


Figure 2: Shows the results from the experiments at 75Hz 1.8V. These were the most promising results of the series of experiments.

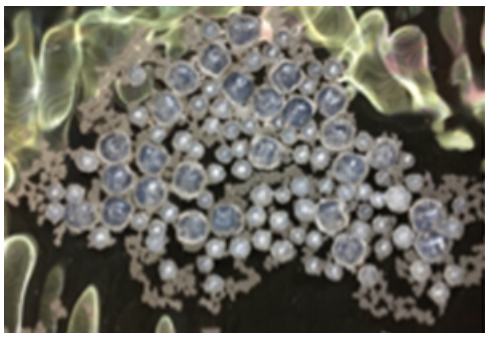


Figure 3: An image showing the clumping ability of the plastics after being exposed to a Faraday wave.

Microscope Test

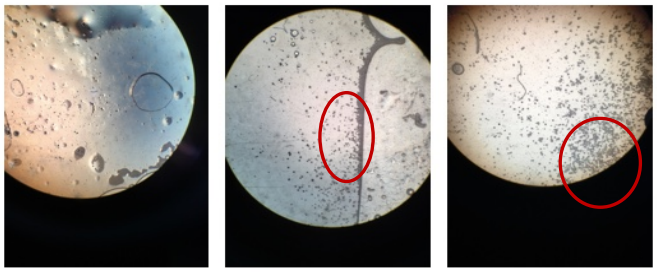
In order to prove that microplastics were attracted to the nurdles, they were placed in solution and then under a microscope. Plastics were put into the 150x150x2mm dish, which filled with water and silicon oil (20cst). The speaker was set to 75Hz 1.5V. After a minute, a nurdle was removed with tweezers and any debris was washed off with ethanol onto a microscope slide. This experiment was repeated again but for one hour. A solution that had not been subjected to a Faraday wave was a control. When comparing the images, it was found that there was significantly more PMA and microfibers when exposed to a Faraday wave.

Filter

When designing the filter, multiple chambers were tested. The dish with two chambers was unsuccessful as the microplastics flowed freely between the two.

There was one more chamber added, which proved more successful. The microplastics would mainly stay in the first chamber, and if they got into the second chamber they immediately rafted at the corners. PMA did not obey this but the nurdles helped reduce PMA movement. The amount observed in the third chamber was reduced when they were introduced into the first chamber when the experiment was repeated.

The basic filter [Fig.3], used the idea of three chambers, with nurdles incorporated into the design, that would be cleaned with ethanol at regular intervals. When trialling the filter, it would only work for a few seconds before the water was drained, which was due to a fault with the pump. The diameter of the tubed used was also a problem, as both poly(ethylene-co-acrylic acid) and poly(ethylene-co-ethyl acrylate) would get stuck in the tubes, which caused disassembly of the apparatus. If this was repeated in the future, a protective gauze should be put over the tubes, to stop any plastics from getting stick, or a tube with smaller diameter should be used.



Images show differences between the debris on the nurdle. The pictures show a nurdle in solution for 1 minute, a nurdle in solution for 1 minute with a Faraday wave, and a nurdle in solution for 1 hour with a Faraday wave.

Using a larger dish would have made tracking and observing the plastics easier but as they were 3D printed the size was restricted. In addition, it would be beneficial to have the chambers covered as there were many leaks from the top of the dishes, and the tubes had a tendency to emerge from the water, even when they were tied down.

Figure 3: Basic filter diagram showing how the plastic moved; nurdles (blue), microbeads (yellow). Chambers were vibrated so a Faraday wave will appear. Size of chambers was altered after further testing. Overall size of the chambers did not matter, there only needed to be smaller channels between them.

Conclusion

In conclusion, there is evidence to suggest that the Faraday waves did affect the behaviour of the microplastics, particularly poly(ethylene-co-acrylic acid) and poly(ethylene-co-ethyl acrylate). From the results, it is clear that at higher amplitudes, there was an increased clumping rate. Although it could not be predicted which corner the plastics would raft in, the plastics would all move to one.

A possible explanation for this is increased electrostatic forces between the particles within the plastics. During the investigation it was found that combining the different plastics would increase the rate of clumping. This was especially true for the PMA powder, which otherwise, was unable to be collected and would fractal across the surface of the solution. The filter design was a good first attempt but there would need to be further work and investigations on it for more reliable results. Regardless, the experiments led to a basic filter design, which will hopefully provide a foundation for further, more detailed, research into the interaction between Faraday waves and microplastics.

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UgSJ Undergraduate Science Journal University of Exeter

Forgotten Patients of the Opioid Epidemic

Elayne Innes

26th October 2017, President Trump declared a national public health emergency in regard to the opioid crisis in America (NBC News, 2017). The President and First Lady's speech was conveyed energetically, driving the masses towards a war on drugs. Nevertheless, the address did not accurately depict the complexity of the opioid crisis. Concentrating on the expanding prevalence of addiction and overdose deaths in the populous. President Trump disregarded the critical distinction between the demographics within the epidemic. Namely, individuals that are addicted to opioids and chronic non-cancer pain (CNCP) patients who are dependent on opiates for pain relief. These distinctions are significant when creating guidelines that impact the accessibility and quantity of medicine available to the patient (Dowell et al, 2017) (Schatman and Ziegler, 2017).

In an editorial from the Journal of Pain Research, Schatman et al have highlighted the failure of the Centers for Disease Control and Prevention (CDC) portrayal the complexity of the opioid epidemic. The most critical issues are the lack of distinction between legitimately and illicitly obtained opioids and their association with the increase in unintentional overdoses and death. The conflation of legally and unlawfully obtained opioids results in analysis skews the data. The CDC proclaims approximately 70% of drug overdose deaths involve opiates; this includes prescription opioids, illicit opioids and illicitly manufactured opioids (CDC, n.d.). Of the 70%, about 35% were attributed to prescription opioids (CDC, n.d.). With the conflation of definitions, 35% suggests the majority of CNCP patients abuse their medication. The average percentage of CNCP patients that become addicted is only 3.27%. (Fishbain et al., 2008).

The knock-on effect of the skewed data affects the design and development of guidelines and policies. Guidelines become bias towards the drug as opposed to the different populations that use the drugs. It is apparent from the President's speech that the CDC's guidelines will impact opioid prescription for pain management. The recommendations are being used to develop new strategies to fight the war on drugs, making it more challenging to obtain opioids on prescription (NBC News, 2017). Praise should be given to Schatman et al for highlighting this issue. Nonetheless, although the authors have recognised the increase in unintentional overdoses and the contributions from both populations within the epidemic, they have neglected to refer to the pharmacogenomic influences that affect this outcome.

For example, the prodrug codeine is a regularly prescribed opioid analgesic to CNCP patients. Codeine is bioactivated via O-demethylation by cytochrome P450 2D6 (CYP2D6) activity into its active metabolite, morphine. The highly polymorphic CYP2D6 gene has varying effects on drug metabolism. Pharmacodynamic and pharmacokinetic studies have distinguished clear CYP2D6 metabolizer phenotypes. These are poor metabolisers (PM), intermediate metabolisers (IM), extensive metabolisers (EM), or ultra-rapid metabolisers (UM) (Crews et al., 2014). PM have a decreased enzyme activity resulting in little or no morphine generation decreasing the analgesia (Mikus et al., 2017) (Lötsch et al. 2009) (Eckhardt et al. 1998).
In contrast, UM produce an increased level of morphine resulting in toxicity at low doses causing life-threatening side effects (Kirchheiner et al., 2007) (Gasche et al. 2004) (Kelly et al., 2012) (Ciszkowski et al., 2009) (Dalén et al., 1997). The constancies of research have enabled guidelines to be created. The Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines recommends guided therapeutic and evidence-based suggestions for individualising treatment with codeine, by CYP2D6 genotyping (Crews et al., 2014).

The analgesic effect of opioids come from the pharmacologically active ingredient (e.g. morphine) binding to the μ1 opioid receptor. The OPRM1 gene codes these receptors. This gene contains a functionally significant variant Chr6(GRCh38):g.154039662A>G (rs1799971). This single nucleotide polymorphism (SNP) causes an amino acid exchange, Asn40Asp. It influences the expression and binding potential of the receptor. The distinct genotypes; AA homozygous, AG heterozygous, GG homozygous determines the functionality of the receptors (fully functional, partially functional, and dysfunctional). Therefore, patients' genotype can influence the analgesic response to the drug (Crist and Berrettini, 2014) (Mura et al., 2013).

It is clear from the current research that genetic polymorphisms affect the pharmacodynamics and pharmacokinetics of opioids 7,8,17,9–16. A substantial amount of research has demonstrated specific genes are associated with the susceptibility to opioid addiction (for more information, read the recommended review by Mistry et al). In a large case-control study, genes encoding the opioid receptors (OPRM1, OPRD1 and OPRK) were investigating the association for opioid dependence risk factors. The study cohort included 1459 heroin-dependent patients,

1495 non-dependent controls and 531 controls from economically disadvantaged neighbourhoods. The study found two OPRD1 SNPs (rs2236857 and rs581111) GA haplotype had associations with a higher chance of addiction. However, no support involving the other genes were found.

Moreover, encouraging research indicates the potential use of an algorithm that integrates genetic and non-genetic factors in determining a patient risk of opioid addiction. A 2017 multi-centre, observational study (Ramsay et al., 2017). evaluated the effectiveness of the Proove Opioid Risk (POR) algorithm to identify opioid disorders in patients correctly. The algorithm was utilised in 186 participants: 94 patients addicted to opioids and 92 healthy patients. The result indicated the algorithm correctly identified addicts 97% and demonstrated to have a sensitivity of the 98% and specificity at 100%. It may be a solution that could be clinically executed pre-emptively to anticipate patients at high risk of addiction before prescribing opioid medications.

President Trump's declaration of a "war on drugs" fails to recognise the needs of CNCP patients fully. Due to this these patients are suffering in response to the change in rules and regulations. Protecting these CNCP patients, two factors must be resolved; first, a distinction between legitimately and illicitly obtained opioids and their association with the increase in unintentional overdoses and death must be investigated. Moreover, secondly, more research is needed for an in-depth understanding of genetic factors that impact the pharmacodynamics and pharmacokinetics. In addition to the association of particular genes to an increased risk of addiction. By implementing genetic testing and algorithms such as POR within the clinical setting, genetic profiles can be developed. It would assist clinicians in choosing the right drug at the right dose. Additionally, more education is required for the public and media with the goal to distinguish between the demographics. It is allowing for the development of non- biased guidelines to be drawn up. It will hopefully give the forgotten patients a voice in this epidemic.

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mpMRI improves the diagnosis of clinically significant prostate cancer: is its use in widescale screening indicated?

Ziad Zeidan

Abstract

Evidence for the mortality benefits of prostate cancer (PCa) screening using prostate specific antigen (PSA) is controversial; longitudinal studies have not demonstrated a net mortality benefit for its use. However, the current evidence does not account for the development of multiparametric magnetic resonance imaging (mpMRI) for diagnosis. Current practice involves conducting systematic transrectal ultrasound guided (TRUS) biopsy, following referral to specialist centres. However, utilising mpMRI prior to biopsy has a significantly improved sensitivity and negative-predicted-value (NPV) for clinically significant disease, as opposed to conducting systematic TRUS biopsies, without prior imaging. Trial data has demonstrated that this practice reduces the diagnosis of insignificant tumours that would otherwise have not caused significant morbidity for patients; thereby, reducing their exposure to further unnecessary treatment. However, heterogeneity in practice across urology centres and the limited inter-reader reproducibility of mpMRI images are major limitations to its widescale use.

Introduction

Prostate cancer has the highest mortality in the developed world out of all cancers (Ferlay et al, 2014). However, the dilemma of whether to screen for and treat the condition remains controversial, with no mortality benefit proven between active monitoring or treatment (Hamdy et al, 2016). Two landmark trials, however, in the USA and Europe assessed the mortality consequences of screening using the PSA test, with the former revealing no significant difference in mortality after 13 years follow-up (Hayes and Barry, 2014; Pinksy et al, 2016). This result has been interpreted with caution, as a significant proportion of the control arm had received a PSA test; consequently, limiting the applicability of the data comparing PSA testing to non-PSA testing (Pinksy et al, 2016). The European trial concluded, however, that the relative risk reduction in PCa mortality with screening was 27% (Schroder et al, 2015). Nevertheless, an additional 27 men needed to be diagnosed with PCa to prevent one death, while an additional 781 men needed to be screened to prevent one death (Schroder et al, 2015). In view of these results, the European Association of Urology (EAU) does not recommend PCa screening (EAU, 2018). Nevertheless, these three studies did not account for the development of mpMRI, a novel high-resolution MRI used notably for visualising prostate malignancies (Ahmed et al, 2017). It can more accurately visualise malignant changes, compared to standard imaging. This review investigated the impact of mpMRI on PCa diagnosis.

According to NICE guidelines, a referral for a prostate biopsy is based on serum PSA and an abnormal digital rectal exam (DRE) upon request by patients (figure 1) (NICE, 2014). No threshold currently exists for an abnormal PSA, as it is not a sensitive or specific marker of significant disease, hence why it is used only on request and not screening (EAU, 2018). Systematic biopsies are recommended via TRUS, in the event of elevated PSA and abnormal DRE, mpMRI is then offered to men with a negative biopsy and a high suspicion of PCa (NICE, 2014). Since the release of these guidelines, mpMRI has been staking its claim as an accurate triage test prior to biopsy to assess the risk of PCa, using a Prostate Imagining Reporting and Data System (PIRADS) score, to guide further decision making (EAU, 2018).

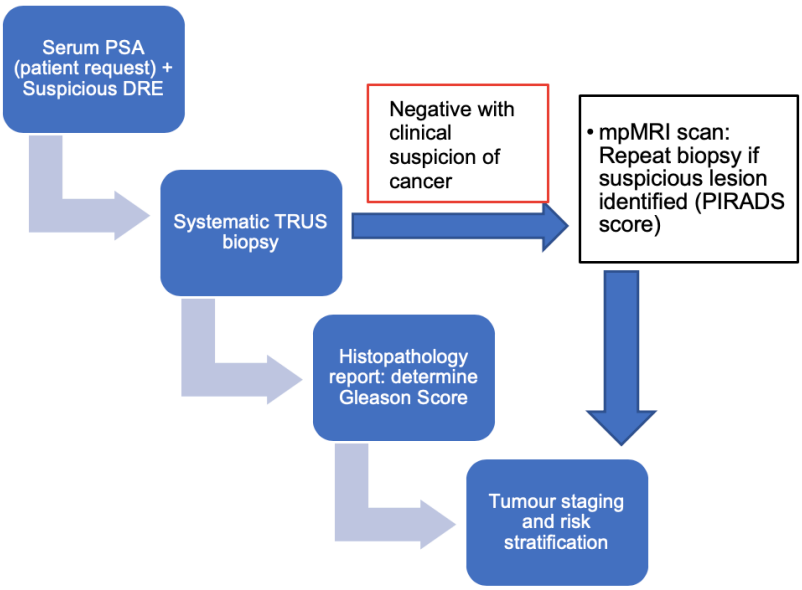

Figure 1. Summary of pathway to prostate cancer diagnosis, as per current NICE guidelines (released in 2014). The guidelines state that a systematic transrectal ultrasound guided (TRUS) biopsy is to be performed with a PSA and digital rectal exam (DRE) suspicious of prostate cancer. The guidelines recommend mpMRI imaging in patients with a negative biopsy and high suspicion of cancer. Nevertheless, a repeat systematic biopsy is not recommended if the scan reveals no suspicious lesions. The use of mpMRI imaging has changed since the release of these guidelines. (National Institute for Health and Clinical Excellence (NICE) (2014)

The use of mpMRI has been proposed to accurately rule out significant disease, as per the PROMIS trial (Ahmed et al, 2017). The results of the trial were supported by a systematic review analysing the sensitivity of mpMRI in detecting clinically significant prostate cancer (csPCa) in men who have not had a prostate biopsy (Fütterer et al, 2015). In addition, two meta-analyses further highlighted the sensitivity and NPV of mpMRI and the PIRADS score (Hamoen at al, 2015; Moldovan et al, 2017).

The role of mpMRI in PCa diagnosis now enables clinicians to re-address the subject of screening. Routine use of mpMRI has been shown to be cost effective, based on modelling of the PROMIS trial (Faria et al, 2018). This raises the question of whether screening can be offered to males with the additional capacity of mpMRI to rule out significant disease. However, low reproducibility of mpMRI images is a major limitation to its widescale use, as interpretation of images is more complex; thereby, requiring specialist experience in analysing prostate malignancies. Furthermore, studies analysed in systematic reviews were markedly heterogenous, due to the variations in practice across centres.

Methods

The NICE and EAU guidelines for prostate cancer were first analysed, following specialist guidance. A review of the literature was then conducted, using both Pubmed and TRIP databases. The following search was conducted: "prostate cancer" AND "MRI" AND "systematic biopsy". Preference was given to identifying randomised controlled trials, systematic reviews and meta-analyses, while the results were analysed for relevance to this review.

Results

This review analysed four studies investigating the impact of mpMRI as a diagnostic tool in PCa. The first was the 2017 English PROMIS trial, a paired-cohort study of 576 men investigating the difference between mpMRI and systematic TRUS biopsy, using template prostate mapping as a reference (Ahmed et al, 2017). As illustrated by table 1, the sensitivity of mpMRI (PIRADS score ≥3/5) in detecting PCa with Gleason Score (GS) ≥4+3, ≥3+4 or ≥7 was 93%,87% and 88% respectively (Ahmed et al, 2017). The Gleason score is given following histological analysis of biopsy specimens, for the purposes of grading PCa (Ahmed et al, 2017).

Table 1: Table outlining results of the PROMIS trial of 576 males, comparing multiparametric MRI (mpMRI) against systematic transrectal ultrasound biopsy (TRUS) for detection of clinically significant cancer of various Gleason Score (GS) in terms of sensitivity, specificity and negative predicted value (NPV). A PI-RADs score of 3/5 was used as a marker for suspicious lesion on mpMRI. Both procedures were compared against template mapping biopsy (TPM). (Ahmed et al. (2017))

Intervention		Sensitivity (%)	Specificity (%)	NPV (%)
mpMRI	GS≥4+3	93	41	89
	GS≥3+4	87	47	72
	GS≥7	88	45	76
TRUS	GS≥4+3	48	96	74
	GS≥3+4	60	98	65
	GS≥7	48	99	63

The use of mpMRI was superior to that of TRUS biopsy, with sensitivities of 48%,60% and 48% respectively (Ahmed et al, 2017). Furthermore, mpMRI had a superior NPV of 76% for any cancer with a GS ≥7, as opposed to 63% for standard TRUS biopsy (Ahmed et al, 2017). The PROMIS trial determined that implementation of mpMRI prior to biopsy would have reduced unnecessary biopsy of clinically insignificant cancers by 27% in the cohort (Ahmed et al, 2017). Consequently, by utilising this technology men can avoid invasive and unnecessary biopsies of malignancies that otherwise would not have caused them distress.

The outcomes of this trial were corroborated by a systematic review and two meta-analyses (table 2). The first systematic review analysed 12 studies (1244 men), revealing a sensitivity range of 76-96% for csPCa with a PIRADS score ≥3/5; the NPV range was 63-98% for csPCa (Fütterer et al, 2015). Furthermore, a meta-analysis of 14 studies (1785 men) assessed similar parameters, revealing a sensitivity of 88% and NPV of 58-96% for detecting csPCa (Hamoen et al, 2015). Finally, a meta-analysis of 48 studies, encompassing 9613 males, without prior biopsy, specifically assessed the NPV of a PIRADS ≥3/5 for determining csPCa (Moldovan et al, 2017). The pooled sensitivity of mpMRI for detecting significant disease was found to be 80.2%, while the NPV was determined to be 88.1%, with a prevalence of 32.9% for csPCa (Moldovan et al, 2017).

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Table 2. Table comparing data of three different systematic reviews and meta-analyses of the diagnostic capacity of multiparametric MRI (mpMRI) in detecting clinically significant prostate cancer (csPCa) in terms of sensitivity, specificity and negative-predicted value (NPV). Studies varied for the reference measure used to assess the performance of mpMRI; in addition, the review by Briganti et al (2015) did not exclude for males with a prior negative biopsy. More importantly, significant heterogeneity was observed within each review, due to the lack of a standardised procedure for conducting mpMRI scans. (Fütterer et al (2015); Hamoen (2015); Moldovan (2017))

Study	*n*	Median age (yrs)	Median PSA (ng/mL)	PIRADS cut off	GS for csPCa	Sensitivity (%)	Specificity (%)	NPV (%)
Futterer et al (2015)	1244	62-65	5.1-13.4	≥3/5	Variable across studies	76-96	23-87	63-98
Hamoen et al (2015)	1785	62-65	5.3-10	≥3/5	Minimum score 6-7	88	45	58-96
Moldovan et al (2017)	9613	Not defined	Not defined	≥3/5	≥7	80.2	59.6	88.1

Discussion

The PROMIS trial represented an important study in highlighting the impact of mpMRI in the UK, with a significant sample size. In addition, participants of the trial were fairly homogenous, in terms of investigations received prior to the trial and estimated disease risk (Ahmed et al, 2017). The GS for csPCa was, also, clearly defined in the PROMIS trial. However, the study did not specifically address the overall use of mpMRI prior to biopsy compared to the protocol of systematic biopsy. Furthermore, patient data was not readily available for assessment. Nevertheless, the results of the PROMIS trial were supported by three systematic reviews and meta-analyses (table 2). The first highlighted the performance of mpMRI across a variety of studies and an overall large sample size. However, it was noted that some study participants had had previous negative biopsies prior to mpMRI, which may have skewed the data (Fütterer et al, 2015). In addition, heterogenous criteria for csPCa and different methodologies were reported across different studies, reducing the reliability of the review (Fütterer et al, 2015). The second study, however, used more homogenous criteria for csPCa to assess the sensitivity and NPV of mpMRI, with the results corroborating with the former study (Hamoen et al, 2015). The major limitation noted in this report was the variable reference test used to assess the performance of mpMRI between studies; without a singular reference test, the accuracy of the results is negatively affected (Hamoen et al, 2015). However, the third study, a large-scale meta-analysis, accounted for this by using prostatectomy specimens as a reference (Moldovan et al, 2017). In addition, it clearly defined the GS for csPCa, analysing men without prior biopsy (Moldovan et al, 2017). However, the NPV varied significantly between studies in this meta-analysis, due to the heterogenous nature of patients (Moldovan et al, 2017). In addition, the results were possibly augmented by the analysis of multiple small trials (Moldovan et al, 2017). It was noted across these three large studies that interpretation of mpMRI images had low inter-reader reproducibility, despite the use of the PIRADS scoring system. This may serve as a limitation to the widescale use of mpMRI, especially in centres with less experienced clinicians. Nevertheless, with more widescale use, and sharing of images and data, it may be possible for more standardised methodologies for interpreting images to develop.

Conclusion

Screening for PCa remains contentious, due to the lack of a gold-standard measure of significant disease and limited homogeneity in diagnosis and management. Furthermore, evidence of mortality benefits for treatment are not conclusive. This review identified a high level of sensitivity for csPCa with the use of mpMRI. However, lack of homogeneity in practice across urology centres and lack of inter-reader reproducibility of mpMRI images limits the application of the data on a wide scale. While the use of mpMRI may have small evidence of cost-effectiveness, its use must first display improved outcomes in patients by means of a longitudinal study. Furthermore, more randomised-control trials on a homogenous population of patients is needed to address the comparative outcomes of the PIRADS score prior to biopsy, in comparison to systematic biopsy, as currently advised by NICE. The development of mpMRI, however, has contributed

positively to diagnosis by clarifying, from an early stage, the projected course of disease. However, in its current capacity, is not firmly indicated as a routine test as part of large-scale screening for PCa. Further research will serve to improve the quality of life men, by reducing unnecessary and invasive medical interventions.

Acknowledgements

I would like to extend my deepest gratitude to Mr Malcolm Crundwell (consultant urologist) for their expertise, guidance and support throughout the completion of this report. I would also like to extend my gratitude to the staff of the Urology Department at the Royal Devon & Exeter NHS Trust for their support throughout my time on placement.

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What components in pathogenic bacterial biofilms function to increase biofilm resistance to conventional antibiotic treatment?

Miles Randeria

Abstract

The purpose of this paper is to investigate several key components of pathogenic bacterial biofilms which are responsible for an increased resistance level to antibiotics. The findings of this paper are broken into three functional categories: Communicative, Penetration Resistant, and Structural components. This paper explores autoinducer molecules, the penetration barrier, and the role of polysaccharides in increasing antibiotic resistance. This paper concludes that all of these components have a role in resistance, though the data does illustrate remaining uncertainty, specifically in how and which matrix components mitigate antibiotic effectiveness.

Introduction

The ability to treat bacterial infection in the human body is one which has revolutionised healthcare within the last century (Arias, 2017). After the discovery of penicillin, the first mass-produced antibiotic drug, the primary cause of death changed from communicable disease to non-communicable disease (Arias, 2017). Unfortunately, some kinds of bacteria retain survival strategies causing resistance to antibiotic treatment (Louie, 2012). Certain bacteria may aggregate into large and cohesive matrices called 'biofilms,' which when mature can increase the minimum bactericidal concentration (MBC) of some antibiotics by between 10 and 100 fold more than that required to remove bacterial colonies of the same species which have not formed a biofilm (Moskowitz, 2004).

These sessile bacteria cause threats to immunodeficient patients, as was evidenced by the death of four infants in an Irish Hospital, where biofilm-forming bacteria was ultimately found in the water supply (Walker, 2015). Biofilms also commonly adhere to implanted medical devices and are associated with post-implantation chronic infections (Stewart, 2001). The mechanisms by which bacteria are able to form these conglomerate systems are complex, and involve intercellular signalling, structural coordination, and even selective differentiation to form insulated colonies (Bjarnsholt, 2013).

The biofilm itself is composed of a combination of microbes and hydrated matrix material, or extracellular polymeric substance (EPS) (Whitchurch, 2002). EPS is a combination of proteins, nucleic acids, and exopolysaccharides (Whitchurch, 2002). The role of this hydrated extracellular matrix is to provide mechanical stability, protection, immobilization or adherence, and intercellular communication (Flemming, 2010). A deeper understanding of these components and their functions may enable better treatment for biofilm infection: A pathology lacking a targeted pharmacological antibiotic treatment (Tania F. Bahamondez-Canas, 2018)

Methods

Research selected to answer this question is exclusively from peer reviewed journals, sourced through 'PubMed.' Primary research was also found through the references of secondary research. Research which was not from a reliable institution (i.e. an individual publishing direct to a website) was excluded. Primary sources had to clearly convey effective methodology for review, and present adequate analysis of results. Reviews with findings which could not be corroborated were excluded. Only articles written within the last two decades were considered, to use as recent data as possible. Both primary and secondary evidence was reviewed, in order to analyse experimental results and gain a good understanding of wider subject knowledge. Secondary studies had to be well referenced (>15 references,) and primary studies were required to clearly state their controlled variables and methodologies for review. Materials had to be relevant and contribute to an understanding of the research question. The components investigated are those which seemingly contributed most to biofilm functions.

Results and Discussion

Communication: Autoinducers & eDNA

One of the most interesting methods by which biofilm forming bacteria are able to protect themselves is through intercellular communication. Biofilms are produced by bacteria in response to 'Quorum Sensing' (M.V., 2018).

This is a method of intercellular communication which results from the production of small signalling molecules called autoinducers (M.V., 2018). As more bacteria inhabit an area, autoinducer concentration increases, and local autoinducer receptors reach higher levels of stimulation (Papenfort, 2017). In the case of biofilm production, once these autoinducers reach a "minimal stimulatory threshold" of concentration, "alteration in gene expression occurs" (Miller, 2001). There is even evidence to suggest that differentiation and metabolic changes occur in response to autoinducer stimulation, creating 'resister populations' of dormant bacteria which can reseed colonies, metabolically supported by other bacteria (WL Cochran, 2000).

An exemplary autoinduced effect is found in premature bacterial self-lysis, regulated by a quorum-sensing related molecule: HQNO (Hazan, 2016). The lysis releases eDNA (external DNA) into the biofilm—another critical component for treatment resistance.

eDNA is located in the matrix (Mike Wilton, 2015). It is hypothesized to spread genes conferring antibiotic resistance and instructions for biofilm formation. eDNA has further been shown to create acidic microenvironments within biofilms and directly bind to and sequester aminoglycosides (Mike Wilton, 2015). eDNA can stimulate the production of surface modifications which decrease antibiotic binding sites on microbes (Mike Wilton, 2015). This effect on resistance was tested by treating two biofilm cultures with gentamycin, an antibiotic, where one biofilm was first treated with a DNAse enzyme which degraded eDNA. Significantly lower levels of resistance were found in the treated culture, and the biofilm formed was not structurally mature, illustrating the importance of eDNA in antibiotic resistance (Hazan, 2016).

Penetrative Barrier: Charged Components and External Proteins

While originally thought paramount to resistance, the diffusion barrier is now thought to have a more "limited importance" (Parsek, 2013). Structural hinderance of antibiotic access to bacteria inside the matrix is now widely accepted to vary between antibiotic and species (Rachna Singh, 2016). For example, a meta-experiment found that across species vancomycin had a 57% reduction in diffusion in biofilms compared to non-biofilms, whereas cefotaxime had only an 11% reduction (Rachna Singh, 2016). The variation in results between species and antibiotic may be important to determining the mechanism by which the penetration barrier acts to discourage antibiotic diffusion.

The difference between penetration may be due to the variety of antibiotic structure. Penetration was measured between two antibiotics by attaching a fluorescent tracer molecule to both tobramycin and ciprofloxacin. Ciprofloxacin "quickly penetrated through the biofilm," but tobramycin simply "accumulated at the periphery" (Parsek, 2013). As tobramycin is positively charged, it was hypothesized that it interacted ionically with another component of the biofilm, such as negatively charged oligosaccharides (Parsek, 2013). This finding was further corroborated by the introduction of positive metal ions to compete for binding sites with tobramycin, which increased penetration (Parsek, 2013).

However, theory is not universally accepted. Some less charged antibiotics which also maintain low penetration are an exception to the rule (Jeff N. Anderl, 2000). An additional hypothesis suggests that penetration is reduced because of antibiotic degradation—Possibly due to extracellular proteins such as β-lactamases which reside within the matrix and degrade proteins upon entry. This hypothesis is evidenced by a study which looked at the penetration of ampicillin and ciprofloxacin. Penetration of ampicillin was far greater in a mutant strain of *Klebsiella pneumoniae* which did not produce lactamases (Jeff N. Anderl, 2000). The various hypotheses suggest the true cause behind the penetration barrier may be multifactorial.

Structure: Oligosaccharides

EPS's (Extra Polymeric Substances) provide a critical "first line" of defence against antibiotic attack (Cynthia Ryder, 2007). A component of this protection is found in polysaccharides produced by biofilm forming bacteria (Cynthia Ryder, 2007). A review focused on three particular polysaccharides: Alginate (As seen in fig.1 A), Psl (Pictured in Fig.1 B), and Pel, whose structure is yet undetermined. The review found that they each played unique roles in infection and antibiotic resistance, allowing bacteria to survive an "aggressive microbial therapy" or "robust immune response" (Cynthia Ryder, 2007).

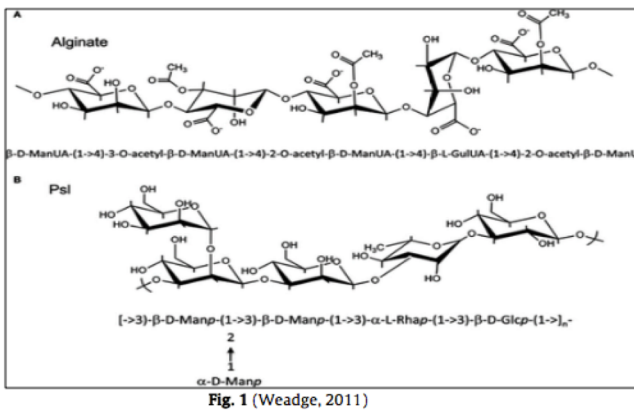

Fig. 1 (Weadge, 2011)

Alginate is problematic in the chronic infection of patients with genetic Cystic Fibrosis (Cynthia Ryder, 2007). Forms of *P. A*eruginosa known as 'mucoid' can over-produce alginate, leading to a dense biofilm matrix which resists antimicrobial use. Alginate was shown in vitro to be over-produced in bacteria with a mutation to the *mucA* gene (F. Heath Damron, 2011). Overproduction has been clearly linked to increased resistance to antimicrobials. A study which compared cultures of *P. Aeruginosa* found that using an alginate lyase enzyme before treatment with *g*entomycin significantly increased the treatment's effectiveness over controls without the enzyme added (MA, 2006).

PSL and PEL are carbohydrates responsible for structure and attachment, and for non-mucoid biofilms. PSL acts predominantly as a "scaffold" which is able to facilitate cell-to-cell adhesion and cell-to-surface adhesion (Cynthia Ryder, 2007). This is critical to the formation of the initial biofilm and keeps bacteria in close enough proximity for effective matrix production (Cynthia Ryder, 2007). The uneven distribution of PSL in mature biofilms also suggests it has a role in biofilm differentiation, as lower concentrations are found in outer areas preparing to detach (Cynthia Ryder, 2007). PEL, while also structurally important, is less explored than its alginate and PSL counterparts (Colvin, 2011). Interestingly, however, PEL specifically has been linked to increased resistance from aminoglycoside antibiotics, by comparing overexpressing PEL cultures against normal PEL cultures (Colvin, 2011). These structural polysaccharides provide challenges to delivery and effectiveness of antibiotic use.

Discussion & Conclusion

As a whole, the question was one which has been well researched and reviewed, particularly within the last two decades. There is a wealth of both primary and secondary studies from reputable sources which corroborated similar hypotheses for biofilm resistance, although many explanations for the mechanisms by which components accomplished these findings are inconsistently evidenced. For example, one study found a relatively fast absorption of tobramycin in early-mature biofilm stages (Cao, 2015). This emphasizes the need for fast treatment of *P. aeruginosa* post-diagnosis but does conflict with other references, including those which are reviewed. Articles are largely peer-reviewed and well cited.

Primary research with large sample sizes compared against controls provide convincing findings regarding resistance mechanisms and roles of matrix components, and although many suggestions for mechanisms were hypothetical the evidence base was large. There was also an abundance of microbiological analysis over a variety of biofilm-forming cultures.

Further research is necessary into the structure of some biofilm components (such as Pel) to develop a better understanding of biofilm specific antibiotic resistance mechanisms. A larger review with more scope than this paper would prove beneficial in further reviewing this subject area.

This paper does conclude that autoinducer molecules, eDNA, extracellular proteins, Alginate, Psl, and Pel are all key components to biofilm antibiotic resistance. The continuation of research into this area may enable novel therapies to combatting these resilient infections.

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Fuelling the First Furnaces: What are Charcoal Remains Telling us?

Hayden Kelly, Oli Marks, Mitchell Sandy, Lucy Farraway, Romily Green, Angus Horne, Nia Morris, Millie Peach

Abstract

An Analytical investigation into the structural properties of charcoal with the aim of identifying an archaeometry method able to determine whether archaeological charcoal has been used in bloomery furnace. Experimental and archarological charcoal samples were analysed using SEM-EDS and CT. It was observed that iron containing deposits had been absorbed onto the surface of used charcoal samples which could have been attributed to slag produced during the smelting process.

Introduction

Analytical imaging and analysis techniques can be used to explore physical evidence of smelting in ancient charcoal remains (Straka, 2017). Techniques used included Scanning Electron Microscopy (SEM), Energy Dispersive X-Ray Spectroscopy (EDS) and Computer Tomography (CT) scanning. Used and unused charcoal were compared to find whether they adsorbed elements during the smelting process, what these elements were and whether they reflect the furnace environment.

Before the introduction of coke and coal fuels, charcoal was the most commonly used fuel for smelting ores. It is produced by slowly burning wood in the absence of oxygen until water and other volatile constituents are removed, leaving the wood to be almost entirely composed of carbon (Browne, no date; Emrich, 1985; FAO Forestry Department, 1987). Charcoal use allows higher furnace temperatures than wood and increases release of carbon monoxide which reduces ore to metal. A characteristic bloomery furnace is shown in **Figure 1**; the action of charcoal in a bloomery furnace is summarised in Error! Reference source not found..

Figure 1: Artistic rendition of a bowl furnace like ones used in Roman Britain

Charcoal is found often during archaeological excavations but not always associated with smelting. To find a fingerprint on charcoal used for bloomery smelting would give archaeologists another tool that they could use to identify bloomery iron smelting sites. For a characteristic marker to be useful, it must be simple to identify and use, must survive archaeological timescales and must withstand conditions in which archaeological samples were found.

Methods and materials

Most archaeological samples studied were obtained at a variety of depths from the archaeological sites across Devon, UK; the experimental charcoal was produced in a bloomery smelt, both provided by the University of Exeter Archaeology Department. These samples were cut into smaller fragments to fit necessary requirements for analysis by selected analytical techniques.

SEM

Vega TC software was used to analyse SEM information. Images were taken at working distance 15mm, minimum magnification, 30kV and saved at scanning speed 6.

EDS

Aztec software was used to acquire energy dispersive spectra over ten small, circular, randomly selected bright spots, and three broader dark areas of background.

Micro-CT

The visualisation program produces a 3D x-ray image which is adjusted as desired to show the sample in its entirety or only the locus of heavier elements.

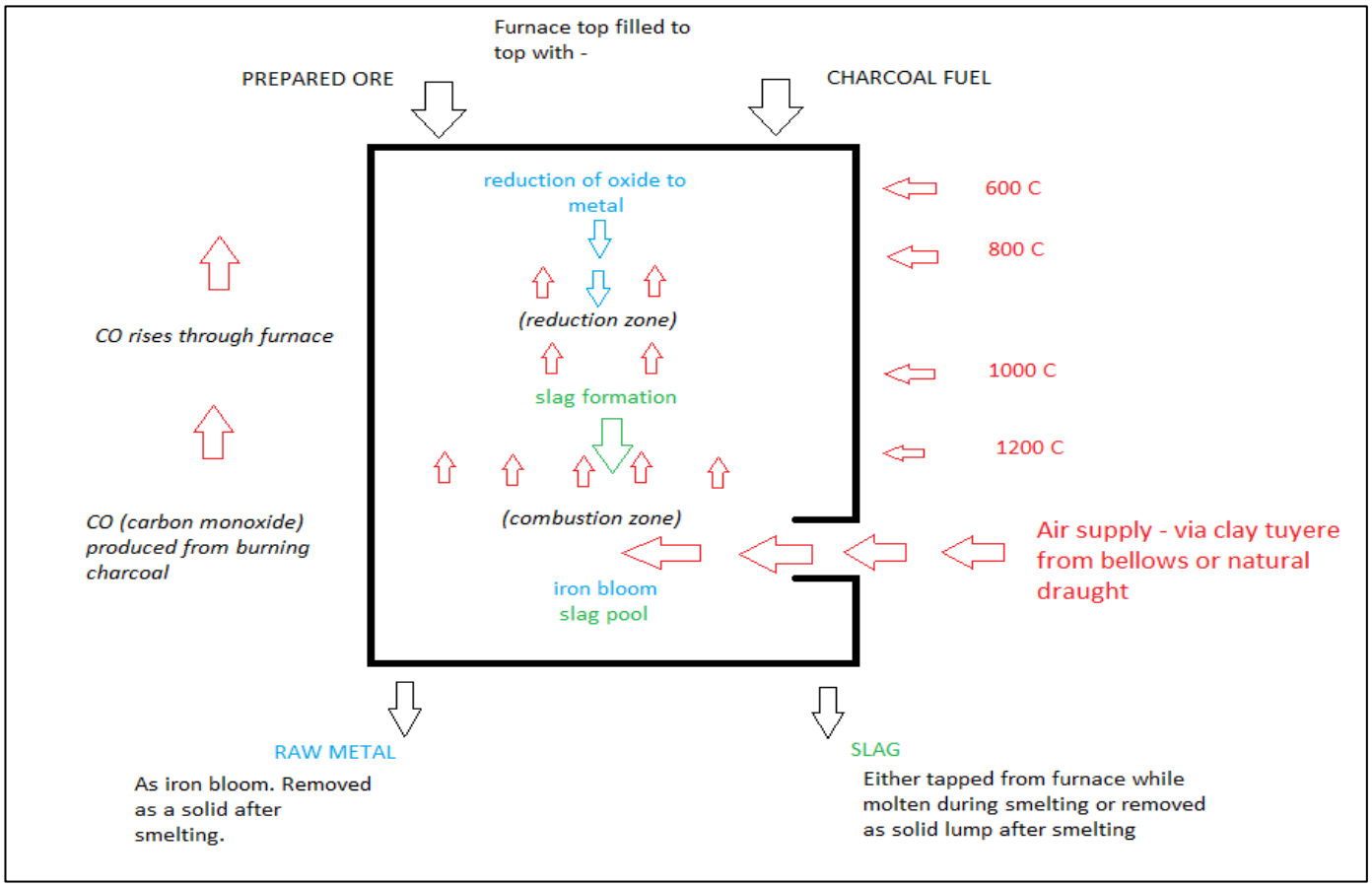

Figure 2. Annotated diagram demonstrating the smelting process within a furnace

Results and discussion

Does the bloomery smelting process physically alter charcoal?

Previous studies (Akinrele *et al.*, 2017; Daykin *et al.*, 2018) have shown that fragment sample analysis yields far better results than powdered sample analysis regarding identifying variation between different samples. The hypothesis that smelting residues adhere to the outside of charcoal pieces was explored using micro-CT. CT is a non-invasive scanning method, allowing clear sample surface and interior observations as shown in **Figure 3**. Unused charcoal (top, **Figure 3**) shows no definitively heavier elements present, suggesting that the charcoal has not been contaminated before the smelting process is carried out. Archaeological samples (seen bottom, **Figure 3**) have heavier materials along the surface of the charcoal and occasionally within interior cracks suggesting possible adsorption of smelted materials. Micro-CT cannot quantitatively say what the surface contaminants are made from, so there is an equal assumption that the material has been deposited from the earth surrounding the buried charcoal, likely mud that's high in variation between different samples. The hypothesis that smelting residues adhere to the outside of charcoal pieces was explored using micro-CT. CT is a non-invasive scanning method, allowing clear sample surface and interior observations as shown in **Figure 3**. Unused charcoal (top, **Figure 3**) shows no definitively heavier elements present, suggesting that the charcoal has not been contaminated before the smelting process is carried out. Archaeological samples (seen bottom, **Figure 3**) have heavier materials along the surface of the charcoal and occasionally within interior cracks suggesting possible adsorption of smelted materials. Micro-CT cannot quantitatively say what the surface contaminants are made from, so there is an equal assumption that the material has been deposited from the earth surrounding the buried charcoal, likely mud that's high in iron.

Brighter areas on backscattered-electron SEM images (indicating heavier elements present) of the charcoal surface were compared to lower brightness areas (indicating lighter elements present) using EDS analysis to characterise elemental composition. Unused charcoal had no significantly bright areas, suggesting no significant adherence of heavier elements.

There is a large standard deviation in iron percentage over all experimental bright spots (standard deviation=20.0%) and all archaeological bright spots (standard deviation=19.4%), as represented by the error bars in **Figure 4**. EDS analysis of the backscatter bright areas on experimental charcoal found a mean content of 30.3% iron.

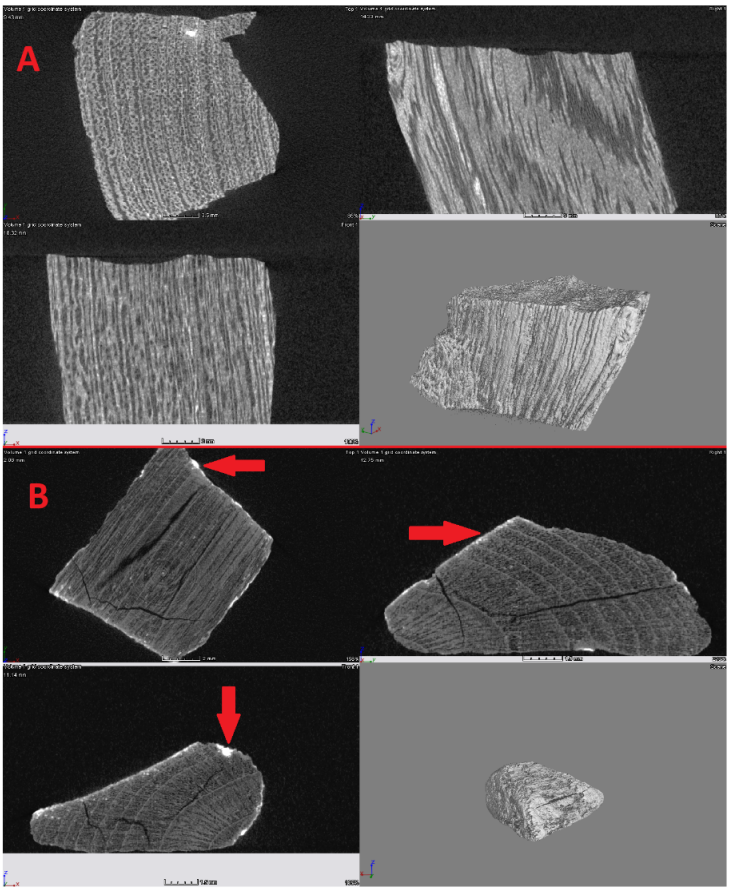

Figure 3 (left): Micro-CT scans of an unused sample of Charcoal (A) and Archaeological sample G1 (B). The lower right section of each sample scan shows overall digital 3D structure while the remaining images are planar scans of the sample interior from each axis. Arrows indicate bright regions where heavier elements have been found.

Does this marker survive the archaeological environment?

Furnaces are located close to historic iron mining sites, in order to reduce transportation needed for bulky, cumbersome ore. The soil around iron veins is often rich in iron compounds and other minerals. As a result, waste bloomery charcoal may have been exposed to iron rich soil for hundreds of years, possibly contaminating charcoal with metal deposits found within the surrounding soil and mud. For this reason it is assumed that Waste bloomery charcoal is often found within waste slag heaps - as would be expected, since it would mean extra processing to separate low value charcoal from waste slag.

Dark areas of archaeological charcoal surface were significantly different to dark areas of experimental charcoal, producing a significant p-value of 0.0332. This can be explained by the larger surface area of dark regions, which makes it more prone to contamination by the soil, and the slag heap. However, the level of iron in bright regions of archaeological charcoal (mean 29.1% iron) is very similar to that of experimental charcoal (mean 30.3% iron). This suggests that the bright spot marker inclusions of archaeological charcoal deposited during bloomery smelting are not significantly affected by archaeological conditions. This is likely due to the concentration of iron being so much higher in the brighter areas that the effect of low background iron concentration in the surrounding soil is much less.

Further characterisation of charcoal surface

Figure 4 also compares the mean level of iron found in different areas of bloomery charcoal to the mean level of iron found in slag associated with such sites. The mean level of iron contained in slag (25.2%, standard deviation=7.7%) is similar to the mean iron content found in bright areas of waste bloomery charcoal. Many of the high-density deposits found on waste bloomery charcoal are very similar in composition to slag, suggesting that during smelting, slag is deposited on charcoal in droplets of varying size and composition.

It is important to note that all data obtained using the SEM-EDS was included in the calculations as the sample size is relatively small. In order to confirm that examination of average iron content of the bright areas on SEM backscatter micrographs acts as a good fingerprint for used and unused charcoal more repetitions of each of the sample must be completed.

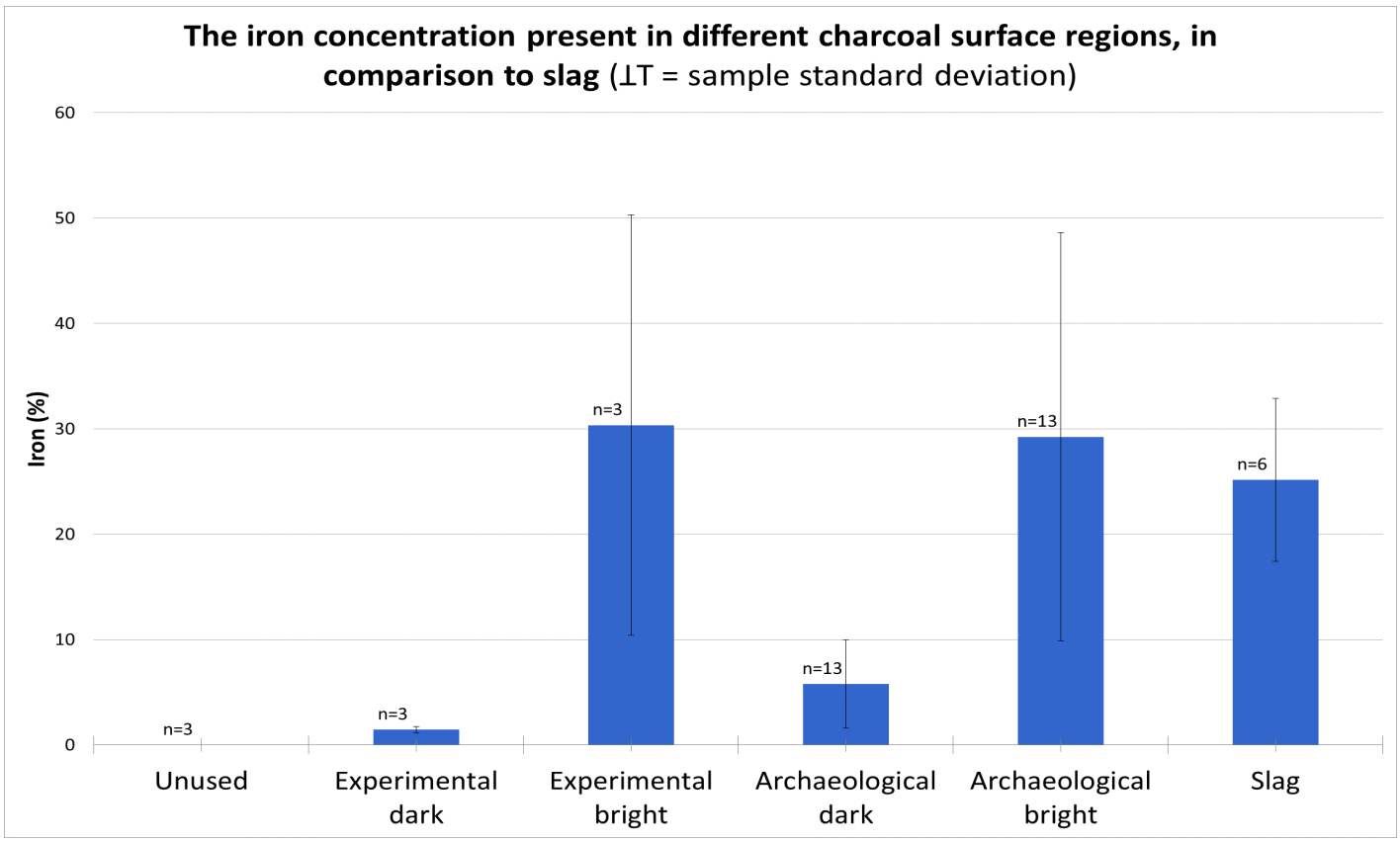

Figure 4:
Comparative bar chart showing mean iron percentage for different regions of charcoal surface (unused, experimental, archaeological), and slag

Conclusions

Statistical analysis of the data obtained in SEM-EDS has allowed us to confirm with a high degree of certainty that bright spots exhibit unique elemental compositions making them interesting potential markers. Specifically, the bright spots were found to be a reliable indicator for usage. If the only information needed is whether the sample has been used, then this alone would suffice. If more information is needed to compare the charcoal to the furnace environment, then SEM-EDS can also provide elemental composition. In particular we found that higher mean levels of iron in charcoal inclusions were indicative of use in iron smelting. This was better than whole surface examination, which would be more prone to the background noise generated by environmental remains deposited by exposure to nature over time. We suggest this could be the basis of a characterisation protocol for use by archaeologists. The images gathered from the CT scanner supports the theory that these deposits of higher molecular weight adhere to the surface of charcoal.
This study did not attempt to characterize/compare the composition of the charcoal surface with the location at which it was taken as supplementary soil samples were not collected. This is a possible area of future study.

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WIRG Experimental Bloomery - Comparison with Ancient Slags.

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Correcting and Perfecting: A History of Plastic Surgery to the Face

Rebekah Lane

Introduction

Plastic surgery, derived from the Greek word, plastikos ('to mould'), defines itself as the practice of reshaping body tissues for reconstructive or aesthetic purposes. Plastic surgery has risen in popularity over the past 20 years, often mistaken as contemporary surgery. However, the beginnings of plastic surgery can be traced back to antiquity (DiBacco, 1994), when facial reconstruction was used after disfigurement from punishment, battle or disease.

With the face being what society appears to judge beauty on, it is understandable why plastic surgery has dedicated much of its development to facial features. However, with approximately one-third of modern plastic surgery focused on cosmetics (DiBacco, 1994), it can be argued that the practice now revolves around making facial appearance look beyond 'normal', into perfection.

This review aims to explore the history of plastic surgery to the face from its origins to modern day, examining advances in skin graft techniques and how the practice evolved to meet the demands of that time.

Method

For this review, a literature search was conducted using the following terms: 'history of surgery', 'plastic surgery' and 'facial reconstruction'. An online archive (Archive.org, n.d.) was used to identify primary sources, including magazine articles and texts written by surgeons from the time. Textbooks and websites covering the history of plastic surgery also aided research. Texts needed to be written in, or translated into, English, and focus on plastic surgery specifically to the face. One television documentary (BBC, 2008) was used to gain a general understanding of plastic surgery techniques used throughout history, however, more reliable sources provided points for discussion.

Discussion

Plastic Surgery in Ancient Medicine

Ancient Egyptians were not known to practice live plastic surgery; however, principles of plastic surgery were often used on the dead in preparation for the afterlife (Random History, 2007). Small bones and seeds were inserted into the nose of Ramses II's mummy, so it remained recognisable in the afterlife (Random History, 2007).

Similar to modern silicone implants, the mummy of Queen Nunjmet had bandages stuffed in her cheeks and abdomen (Random History, 2007). Although the Egyptians possessed dexterity to perform more extreme surgery on the living, their practice was confined to wounds, fractures and dislocations (Ellis, 2009). The first recorded account of plastic surgery to a living face dates back to 1500 B.C. in ancient India. (Hollingham, 2008). Ancient India is considered the birthplace of plastic surgery and boasted facial reconstruction techniques which, at the time, were revolutionary. In 900 B.C, the Hindu surgeon, Sushruta, founded the 'attached-flap method' of rebuilding noses, which was commonly used on those who had lost their nose as a form of punishment for adulterous behaviour (DiBacco, 1994; Saraf and Parihar, 2006). A leaf large enough to cover the severed nose provided template for a patch of skin, usually taken from the forehead or cheek. Scarifying with a knife, the flap of skin would be brought from its original site, twisted round, and moulded over the hole left from the amputation to create a nose (Kaviraj Kunja Lal Bhishagratna, 1916). The Indians understood the importance of preserving blood supply, so the flap would remain connected to the original location by a pedicle (Hollingham, 2008). This method of nasal reconstruction, known as the 'Indian Method of Rhinoplasty', was kept secret for centuries (Haiken, 1999).

Revelation of 'the flap method' in Europe emerged after being published in the 'Gentleman's Magazine of Calcutta' in October 1794 (The Gentleman's Magazine, 1794). The article reported the story of a prisoner of war, whose disfigured nose was reconstructed by an Indian man. Witnessed by British surgeons, they described a procedure similar to Sushruta in 900 B.C., cutting a flap of skin from the forehead to create a nose. Replacing the leaf, the template was formed from a thin plate of wax (The Gentleman's Magazine, 1794). This method is shown in Figure 1. This long-practised operation was generally successful, producing noses that were secure and closely resembled a natural nose (Hollingham, 2008). This breakthrough in skin grafting was utilised by plastic surgeons well into the nineteenth century, including pioneering Scottish surgeon, Robert Liston (Liston, 1840).

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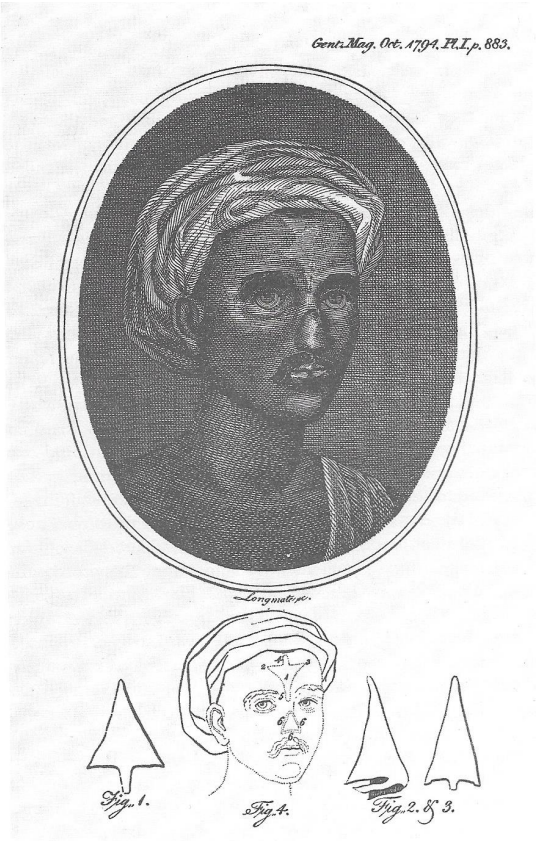

The image of Cowasjee in an article authored by "B.L." in the *Gentlemen's Magazine,* London, 1794. This article marked the reintroduction of rhinoplasty into Europe.

Figure 1. The 'Indian Method of Rhinoplasty' Gilman, S.L. 1999. Making the body beautiful: A cultural history of aesthetic surgery. United States. Princeton University Press. Originally appeared in Nichols, John. 1794. The Gentleman's Magazine.

The Progression of Plastic Surgery

In Renaissance Italy, the syphilis outbreak created a demand for new noses and drove the progression of plastic surgery. Gaspare Tagliacozzi, desperate to help those severely affected by syphilis, became a pioneer to surgery of 'defective parts' (Hollingham, 2008). His aim was to reconstruct noses using a flap of skin taken from the forearm. After incising a flap of skin to create the nose, the patient would raise their arm to allow the flap to be sutured to the face. Keeping the flap attached to the patient's arm allowed continuous blood supply, thus preventing necrosis and reducing the risk of infection. Once the new nose developed its own blood supply from the face, the flap was then cut from the arm and Tagliacozzi would sculpt the skin to resemble the shape of a nose (Gilman, 1999; Hollingham, 2008; Ellis, 2009). A downfall of this procedure was that it required the patient to hold their arm to their face for two weeks. Therefore, Tagliacozzi would strap the patient's arm in place using a headpiece, an impractical design leaving the hand resting on the head and elbow protruding in front, shown in Figure 2 (Gilman, 1999; Hollingham, 2008). This was a small price to pay for the prospect of a new nose.

However, Tagliacozzi's advances in facial reconstruction were short-lived. His practice was disapproved by the Catholic Church, who accused him of interfering with God, and many surgeons believed his technique was too hazardous, and hence continued to favour the Indian method (Hollingham, 2008; Ellis, 2009).

Using flaps to reconstruct the nose continued into the nineteenth century. The discovery of anaesthetic in the 1840's (Ellis, 2009) revolutionised surgery as the public began electing themselves for operations. Plastic surgery was amongst the most popular surgical practice as people went to surgeons with a determination to look more beautiful (Hollingham, 2008).

Figure 2. Tagliacozzi's headpiece for nasal reconstruction. Photograph taken from Hollingham, R. 2008. Blood and guts: A history of surgery. United Kingdom. Ebury Press. Originally provided by the Wellcome Library, London.

In the early twentieth century, surgeons moved away from the knife and began inserting substances beneath the skin. Surgeons attempted to reshape noses using ivory, metal, celluloid, and gutta percha (Kolle, 1911; Hollingham, 2008; Paget 1902). However, many of these attempts to beautify patients failed until the discovery of paraffin wax. Surgeons would melt the paraffin wax, inject it into the nose, and mould a new shape as the wax set (Kolle, 1911; Hollingham, 2008). Several cases showed this method of rhinoplasty to be successful and, unlike surgery, could be accomplished in only a few minutes and would leave no scar (Paget, 1902). However, this practice provides us with one example of how society's desperation for beauty can devastatingly backfire. Paraffin wax injections led to adverse effects that not only distorted the face further from perfection, but also proved fatal. Dubbed "wax cancer", lumps of wax from the nose accumulated in other areas of the face, thus undoing the surgeon's work and causing infection and muscle degradation (Santoni-Rugiu and Sykes, 2007; Hollingham 2008). Paraffin clots found in the bloodstream were responsible for causing fatal strokes and heart attacks (Kolle, 1911).

Faces of War

The technological advances in weaponry that accompanied WWI brought injuries that were previously unimaginable and thus forced surgeons to innovate their techniques. Surgeon, Harold Gillies, was shocked by the inadequacy of British plastic surgeons to correct the damaged faces of soldiers (Hollingham, 2008). With the opening of Queen's Hospital in Sidcup, the first of its kind dedicated to plastic surgery, Gillies began revising various methods in preparation for the ravages of war (Gillies, 1920).

Facial disfigurement and gaping noses were amongst common injuries faced by soldiers. Focused on rebuilding authentic noses for those who lost them, Gillies returned to the favoured (Indian) flap method, adapting it to include rib cartilage for structural support. The cartilage was initially implanted into the forehead and left to heal for several weeks. The flap of skin and cartilage was then twisted downwards and placed over the hole to form a new nose, once again, creating a pedicle to maintain blood supply. In the weeks following, excess skin was removed, and a new nose was shaped, deeming the operation to be successful (Gillies, 1920; Hollingham, 2008: Ellis, 2009). However, Gillies became dangerously ambitious in obtaining a perfect result, demonstrated in the case of an aircrew Lieutenant who suffered full facial burns in a plane crash. An attempt to replace the whole face using a chest flap lead to graft infection, and regrettably the patient died of heart failure just 24 days later (Hollingham, 2008).

Despite early failures, Gillies began to perfect a method of facial reconstruction known as the Russian tube pedicle. Flaps of skin obtained from the arm, cheek, forehead or chest were folded to make a tube, enclosing the blood supply and reducing exposure to infection. These tubes were then used to reconstruct new noses and jaws (Gillies, 1920; Hollingham, 2008; Ellis, 2009). This technique is shown in Figure 3.

For patients who suffered upper body burns along with facial disfigurement, the option of creating a skin flap from nearby tissue was unfeasible. To overcome this, Gillies experimented with innovative techniques and introduced his original waltzing pedicle (Hollingham, 2008). Tube pedicles could be taken from the leg and swung up the body in stages before being attached to the face. This ingenious idea meant skin grafts could safely be taken from any part of the body, a concept that remained in general use until recent years (Hollingham, 2008; Ellis, 2009).

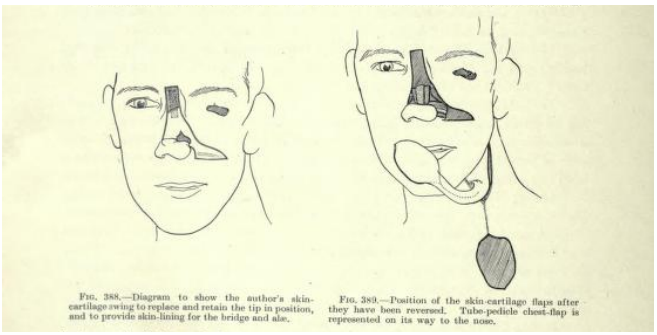

FIG. 388.—Diagram to show the author's skin-cartilage swing to replace and retain the tip in position, and to provide skin-lining for the bridge and alæ.

FIG. 389.—Position of the skin-cartilage flaps after they have been reversed. Tube-pedicle chest-flap is represented on its way to the nose.

Figure 3. Diagram illustrating the 'Tube-pedicle chest-flap' for nasal reconstruction, designed by Harold Gillies. Gillies, H.D. 1920. Plastic surgery of the face based on selected cases of war injuries of the face including burns, with original illustrations. London. Frowde.

Conclusion

The progression of plastic surgery throughout history was essentially driven by the needs of the patients at the time. Surgeons of the past endeavoured to repair the faces of those victim to punishment, disease, or war, using innovative techniques to create new facial features close in resemblance to the original. However, there is greater pressure on the plastic surgeons of modern medicine as patients demand perfection and aspire for beauty, not just normality. But perhaps the advancement of plastic surgery as a speciality will be driven by this pressure.

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Can the UK take note of Cuba's Social Values to save the NHS?

Jenna Hussain

Introduction

It is no secret that currently the U.K. healthcare system is under a magnitude of pressure. Both financially and politically, it is under great strain to keep up with increasing demands and expectations, with little extra resources supplied to help lighten the load. One such challenge facing the National Health Service (NHS) here within the U.K. is the demographic shift towards an ageing population. (Office for National Statistics, 2019)

With an ageing population comes a plethora of challenges and obstacles for healthcare systems. One such pressure includes the rising prevalence of non-communicable diseases such as dementia, cardiovascular disease and diabetes (Bosch-Bayard et al., 2016). This essay will focus on the impact of the increasing prevalence of dementia on healthcare systems and go on to discuss how the U.K. can mitigate this rising pressure by learning from strategies adopted in Cuba.

Analysis

The U.K. is in the midst of demographic shift. Although the predicted average life expectancy has recently stalled according to the latest data, those born between 2015-2017 are, on average, expected to live for ~79.2 years, if male, and 82.9 years, if female - this represents an increase in life expectancy of 1.4 years from those born between 2005-2007(Office for National Statistics, 2019). Further highlighting the trend, approximately 18.2% of the UK population were aged 65 years or over in 2017, compared with 15.9% in 2007; this is projected to propagate to 20.7% by 2027 (Office for National Statistics, 2019). Although this can be interpreted as reflecting the positive impact of developments within medicine and healthcare, this ageing shift further increases the pressure on healthcare systems such as the U.K.'s.

Ageing populations experience a rise in prevalence of a range of age-related diseases - one of the most burdensome being dementia (Bosch-Bayard et al., 2016). The current cost of dementia on the NHS is estimated at £26 billion a year, this is projected to more than double to £55billion by 2040 (Prince et al, 2014).

Dementia is an overall term given to describe a category of diseases associated with a decline in cognitive function (i.e. short-term memory loss, decline in reasoning and judgement etc.) and can manifest other symptoms such as hallucinations and changes in personality. Alzheimer's Disease and Dementia, 2019). Over recent years, dementia has become a major public health issue due to the heavy burden it places not only on patients and their families, but also on healthcare resources and funds (Langa et al., 2001). This is highlighted in how at the end of 2012, dementia was reported to have had a higher combined health and social care cost on the NHS (£11.9billion) than cancer (£5.0billion) and chronic heart disease (£2.5billion) combined (Luengo-Fernandez et al, 2015).

It is estimated that the prevalence of dementia doubles with each 5-year increase in age past the age of 60 (Ferri et al., 2005). As a result, it is estimated that the worldwide prevalence of dementia is projected to triple by 2050 from its current prevalence of 50million. (WHO, 2015.) There is currently no established cure for dementia and for this reason, preventative care is the best method of approach. By identifying and thus tackling modifiable risk factors of dementia, we can act to mitigate the rising prevalence. The National Institute for Health and Care Excellence (NICE) has subsequently identified social isolation as a modifiable risk factor for dementia (Livingston et al., 2017) and this can be one focus of action in adopting a preventative approach.

Results from a meta-analysis of 19 longitudinal cohort studies determined there to be an elevated dementia risk for participants with limited social interaction (RR 1•41, 95% CI 1•13–1•75) and those with infrequent social contact (RR 1•57, 95% CI 1•32–1•85) (Kuiper et al., 2015). Although the varying length of study follow-up should be considered when interpreting these results, it is clear to see that when combined with NICE's modifiable risk factors (Livingston et al., 2017), targeting social isolation to alleviate the pressures associated with rising rates of dementia seems the way forward.

Whilst the U.K. is in a very different political and economic position, Cuba's healthcare system shares many parallels to the NHS. Healthcare in Cuba is universal and free. I had the privilege of experiencing this on a recent study trip in December 2017. Engrained in Cuban culture and constitution, healthcare is seen as a basic human right as well as a moral obligation within their society. For this reason, it has been said that Cubans 'live like the poor, and die like the rich' (BBC News, 2019) reflecting their health trends of an ageing population with a disease profile matching that of more developed nations such as the U.K. (WHO, 2015.). As a result of this shift, Cuba has been forced to take on a more pro-active preventative medicine approach to tackle the challenges associated with an ageing population.

Also facing the threat of increasing dementia prevalence, Cuba runs state-funded day-centres as a method to mitigate social isolation; an established modifiable risk factor. These day centers provide a base for those otherwise at risk of loneliness to interact and participate in physical activity, whilst alleviating pressure on carers and social support for that time period. I was able to visit one of these day centres whilst in Cuba. Havana's Casa de Los Abuelos (Home of the Grandparent's) was one of the many day centres located within the city and provided great insight into how a collective community scheme can have such a positive influential impact at both a societal level and at a large state level in regards to alleviating pressure on the healthcare system (see Figure 1). Although accommodation wasn't supplied, meals, health checks and activities were all funded for by the state and most importantly, those who attended were able to participate in socially stimulating activities which they would otherwise not have had. Speaking to many of the day residents gave me great insight into the effectiveness of this preventative scheme. One resident revealed to me that before she started attending the day centres she could sometimes go up to a week without having a conversation with another person; just one example of the degree to which social isolation is endemic amongst the elderly population and moreover how easily it can be alleviated.

Cuba is not alone in this method of social care. The U.K. does fund a small number of non-residential day centres, yet U.K. councils have closed many of these due to lack of funding, resources and uptake (Devon County Council, 2014). Although costing has to be considered in decisions regarding resource implementation, it can be assumed that the cost of funding day centers in the hope they help alleviate even a small percentage of the rising prevalence of dementia is much smaller than the health and social costs that would incur from managing that patient should they go on to develop dementia having not adopted a preventative strategy early on.

Figure 1. Photo taken from Casa de Los Abuelos (14/12/2017) during fieldtrip to Havana. Photo demonstrates the community at the day-centre getting involved in singing and dancing activities as a method of social and cognitive stimulation. Those involved consented to having their photograph taken.

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VOLUME 3 (2018-2019)

UgSJ Undergraduate Science Journal University of Exeter

How has *Pseudomonas aeruginosa* acquired *Klebsiella pneumoniae* carbapenemase (KPC) to resist carbapenem treatment?

Sizar Doski

Abstract

The World Health Organisation has classified *Pseudomonas aeruginosa* among the top-tier critical bacteria that require novel antibacterial therapy. *P. aeruginosa* is an opportunistic nosocomial gram-negative bacterium with the ability to survive in a variety of environmental conditions. It is responsible for increased morbidity and mortality in immunocompromised patients and in patients with cystic fibrosis. It can resist antibiotics through intrinsic and acquired mechanisms – making treatment problematic. There is evidence of horizontal acquisition of enzymes that confer resistance to carbapenems, a last resort gram-negative bacteria treatment. Particular focus was on the *Klebsiella pneumoniae* carbapenemase (KPC) which is characteristic in carbapenem-resistant *Enterobacteriaceae*. The KPC-2 enzyme in *P. aeruginosa* has recently been isolated in Europe. Some novel potentiators tested in conjunction with carbapenems have fallen short of initial expectations. The novel β-lactamase inhibitor relebactam with imipenem, a carbapenem, exhibits promising results. Further research is essential to prevent a potential multi-drug, or even pan-drug, resistant *P. aeruginosa* epidemic.

Introduction

Carbapenem resistant *Pseudomonas aeruginosa* along with *Acinetobacter baumannii* and carbapenem-resistant *Enterobacteriaceae* (CRE) was specified by the World Health Organisation (WHO) list for bacteria with critical priority for novel antibacterial development (Tacconelli et al., 2018).

Multi-drug resistant (MDR) strains of bacteria are a burden to healthcare systems since it is associated with prolonged hospitalisation, morbidity and mortality (Carceo et al., 2016). This is particularly the case for nosocomial pathogens, such as *Pseudomonas aeruginosa,* which may compromise advanced medicine such as surgery and treating immunocompromised patients (Potron et al., 2015).

Pseudomonas aeruginosa is an asporogenous, monoflagellated, non-fermenting gram-negative rod bacteria of considerable clinical importance (Potron et al., 2015). It is a ubiquitous microorganism with few nutritional requirements and the ability to survive in a variety of environmental conditions (Wu et al., 2015). *P. aeruginosa* is classified as an opportunistic pathogen, responsible for hospital acquired bloodstream, urinary tract and pulmonary or device related infections, frequently in immunocompromised patients (European Centre for Disease Prevention and Control, 2018). Over 60% of adults with cystic fibrosis have chronic pulmonary infections due to *P. aeruginosa,* which is also associated with increased mortality (Lund-Palau et al., 2016). *P. aeruginosa* has selective ability to prevent antibiotic molecules from penetrating its outer membrane, making it intrinsically resistant to many antibiotics.

In addition, resistance of *P. aeruginosa* can be acquired through a variety of mechanisms including reduced permeability, degrading enzymes, modified antimicrobial targets and efflux pumps (ECDC, 2018). This review will highlight the molecular mechanisms of resistance demonstrated by *Pseudomonas aeruginosa* with emphasis on carbapenems and its acquisition of the *Klebsiella pneumoniae* carbapenemase (KPC). It will also consider current antibiotic treatments and investigate potential novel targets for antibiotic treatment of *Pseudomonas aeruginosa.*

Methods

A literature search using the University of Exeter Electronic Library was conducted with the search terms: '*Pseudomonas aeruginosa*', 'KPC' or '*Klebsiella pneumoniae* carbapenemase'. The same criteria were applied to Scopus, Cochrane and Trip-Database searches. In addition, 'Carbape*, resistant, *Pseudomonas aeruginosa*, treatment' was used to identify carbapenem treatment in Europe. The searches were conducted in November 2018, exclusions based on publication date were not made. Articles were critically appraised using the Critical Appraisals Skills Programme (CASP) checklist.

Results and Discussion

In the EU/EEA, 30.8% of *P. aeruginosa* were resistant to at least one of the antimicrobial groups (Table 1). The highest reported resistance was found in fluoroquinolones (20.3%), followed by piperacillin/tazobactam (18.3%), carbapenems (17.4%), ceftazidime (14.7%) and aminoglycosides (13.2%). There is a clear divide between the West and East of Europe for the rates of resistance to carbapenems, ranging from 0% in Iceland to 53.4% in Romania (European Centre for Disease Prevention and Control, ECDC; 2018).

Table 1. Dissemination of Pseudomonas aeruginosa in Europe. The total number of isolates and resistance combinations against carbapenems, fluoroquinolones, piperacillin/tazobactam, aminoglycosides and ceftazidime (European Centre for Disease Prevention and Control, 2018).

Resistance Pattern	Percentage (%) of total (Number of isolates)
Fully susceptible	69.2 (11691)
Single resistance	
Total	12.5 (2104)
• Carbapenems • Fluoroquinolones • Piperacillin + Tazobactam • Aminoglycosides • Ceftazidime	4.6 (775) 4.1 (696) 2.0 (339) 1.1 (185) 0.6 (109)
Resistance to two antimicrobial groups	7.0 (1185)
Resistance to three antimicrobial groups	4.0 (669)
Resistance to four antimicrobial groups	3.4 (577)
Resistance to five antimicrobial groups	3.9 (659)

Mechanism of resistance

P. aeruginosa exhibits antimicrobial resistance through active efflux pumps, membrane permeability, porin alteration and target modification. Efflux pumps may be involved in natural and acquired resistance following mutations due to antibiotic pressure (Bassetti et al., 2018).

Whereas, the OprD porin is intrinsic and can promote the internalisation of the carbapenems: meropenem and imipenem. An alteration of OprD can overexpress the efflux system in *P. aeruginosa* which confers an intrinsic high level of resistance to many classes of antibiotics, but primarily imipenem (Bassetti et al., 2018). In addition, the four classes of β-lactamases, which inhibits the action of β-lactam antibiotics (Figure 1), are the most important for developing carbapenem resistance.

The four classes of β-lactamases range from Class A to D:

- Class A are extended spectrum β-lactamases (ESBL) resistant to cephalosporins such as imipenem or meropenem.
- Class B β-lactamases, known as Metallo-β-lactamases (MBL), can hydrolyse carbapenems and other β-lactams efficiently (Nordmann et al., 2012).
- Class C β-lactamases can hydrolyse broad and extended-spectrum cephalosporin.
- Class D β-lactamases, known as oxacillinases (OXA), has intrinsic carbapenemase activity (Potron et al., 2015).

CREs mechanism of antibiotic resistance is primarily through the production of carbapenemases encoded on transmissible plasmids (Yawetz, 2018), which can hydrolyse most β-lactam rings including the carbapenem ring. Carbapenem resistance can also arise from a combination of ESBL production and intrinsic outer membrane permeability (Centers for Disease Control and Prevention, 2013).

All the mechanisms for *P. aeruginosa* resistance are summarised in Table 2. The plasmids that attributes to resistance in CRE are predominantly found in KPC, MBL and OXA-48 (Nordmann et al., 2012). In *P. aeruginosa*, carbapenemase properties are exhibited from several Guiana extended-spectrum (GES) and KPC type ESBLs, MBL and OXA (Table 2).

The first GES-type carbapenemase from *P. aeruginosa* was GES-2, a variant that possess an amino acid substitution that broadens its antibiotic resistance from lactamases to hydrolyse carbapenems (Potron et al., 2015). In addition, significant carbapenemase activity has been found in GES-5, and more recently, in GES-18 isolated from Belgium. Oxacillinases that hydrolyse carbapenems include OXA-40 and OXA-198, which shares some amino acid identity with carbapenemase-resistant OXAs reported in other gram-negative bacilli (El Garch et al., 2011).

KPC in P. aeruginosa

Earlier reviews on *P. aeruginosa* reported a rarity in the presence of KPC enzymes. KPC is found on the Tn3-type transposon Tn4401 encoded by the bla_{KPC} gene, with the ability to insert into a huge variety of gram-negative bacteria (Nordmann et al., 2012). Since *P. aeruginosa* usually harbour MBL and OXAs, KPC producing *P. aeruginosa* is remarkable. KPC producing isolates of *P. aeruginosa* were first identified in a Colombian hospital and have over the last decade been reported in other countries such as Brazil, USA and China (Hagemann et al., 2018). Hagemann et al. (2018) reported the first sighting of KPC-2 in isolates of *P. aeruginosa* in Germany, the first identification in Europe. The isolate of KPC-2 in Germany follows reports of KPC-2 producing *P. aeruginosa* in Brazil in 2014. This is in contrast to the statement from Potron et al. (2015), that horizontal transfer of the gene from *Enterobacteriaceae* to *P. aeruginosa* was not evident. Furthermore, the bla_{KPC} gene was associated with the Inc type HI1 plasmid in the study of Hagemann et al (2018). In contrast, a KPC-2 genome sequence study by de Oliveira Santos et al. (2018) found KPC-2 on the IncQ1 plasmid. These are two mobile plasmids which can propagate resistance in cyanobacteria, gram-positive and gram-negative bacteria. Therefore, the potential for continuous spread of the KPC-encoding genes through a variety of transferable plasmids is a significant risk.

Table 2. Adapted from Bassetti et al. (2018) illustrating all the different mechanisms P. aeruginosa possesses – both intrinsic and extrinsic.

Location	Resistance mechanisms	Targeted antibiotics	Type of resistance
Chromosomal (intrinsic)	AmpC–type cephalosporinase	β-lactams	Inactivates antibiotic
	Class D oxacillinase OXA-50	β-lactams	Inactivates antibiotic
	Aminoglycosides inactivating enzymes	Aminoglycosides	Inactivates antibiotic
	Efflux systems (overexpression)	Multiple antibiotic classes – possibly carbapenems (Karampatakis et al., 2018)	Efflux systems
	Decreased membrane permeability	Multiple antibiotic classes – including carbapenems	Membrane impermeability and purines
	DNA gyrase and topoisomerase IV	Fluoroquinolones	Target modification
	LPS modification	Colistin	Target modification
Imported (genetic transfer)	Class A serine β-lactamases (*PSE, CARB, TEM, EM, SHV, CTX-M, PER, VEB, GES, IBC, KPC*)	β-lactams + Carbapenems (GES-2,-5,-18) and KPC (-2,-5)	Inactivates antibiotic
	Class B Metallo-β-lactamase (IMP, VIM, SPM, GIM)	Carbapenems	Inactivates antibiotic
	Class D ESBL (OXA-types)	β-lactams + Carbapenems	Inactivates antibiotic
	Aminoglycosides inactivating enzymes	Aminoglycosides	Inactivates antibiotic
	Ribosomal methyltransferase enzymes	Aminoglycosides	Target modification

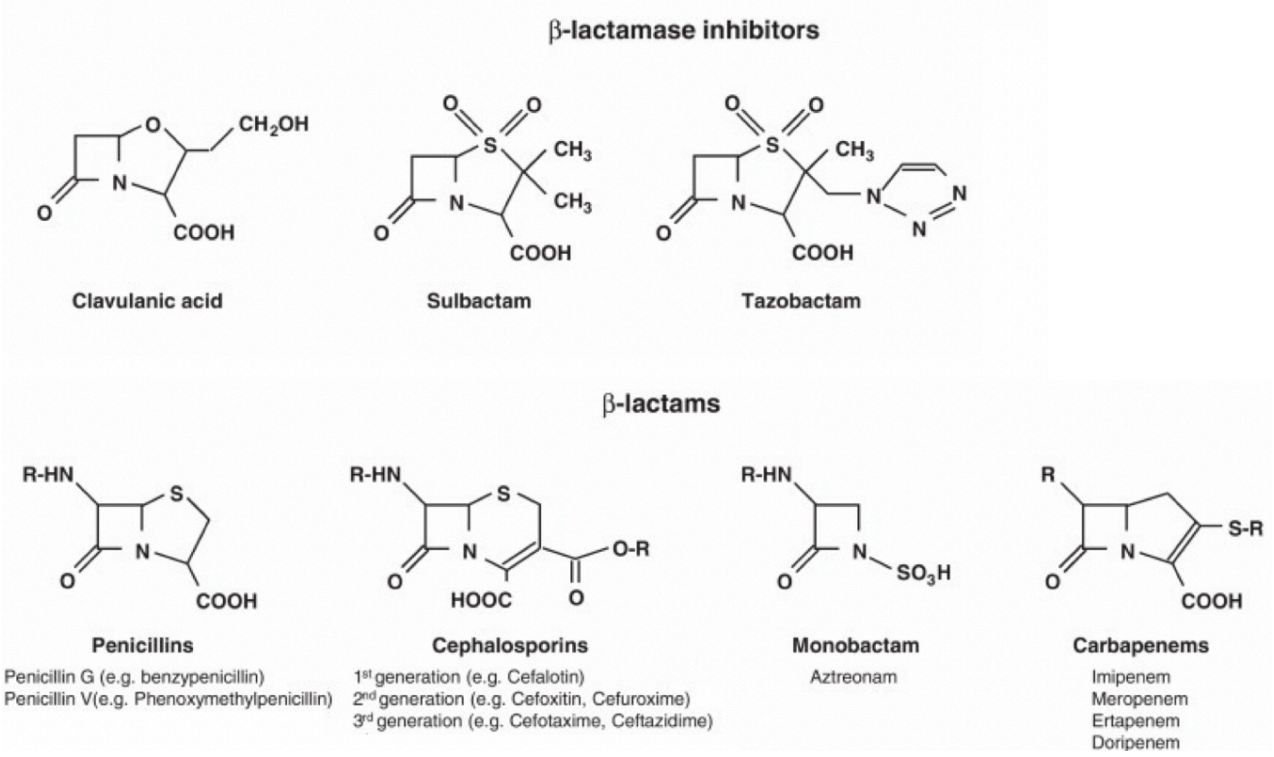

Figure 3. demonstrating the chemical structure of β-lactamase inhibitors and β-lactams – highlighting the β-lactam ring present in both classes (Nordmann et al., 2012).

β-lactamase inhibitors

There are three clinically significant β-lactamase inhibitors (BLIs) that derive from β-lactams with weak antimicrobial activity: clavulanic acid, sulbactam, and tazobactam (Figure 1). The similarity in BLI's chemical structures to β-lactamases allows an interaction with the active site of the β-lactamases secreted by the bacteria, which essentially make BLIs suicidal substrates (Nordmann et al., 2012).

All three of the BLIs poorly inhibit KPC enzymes making them ineffective treatment against CRE infections, including *P. aeruginosa* (Nordmann et al., 2009). In contrast, diazabicyclooctane inhibitors avibactam and relebactam display a broad spectrum β-lactamase inhibitor that covers the KPC enzyme among other β-lactamases, in combination with ceftazidime and imipenem, respectively (Hecker et al., 2015). Vaborbactam is a novel BLI based on a cyclic boronic acid pharmacophore without a β-lactam that acts as serine BLI. The inhibitor was discovered whilst targeting KPC carbapenemase. Thus, the pharmacological, microbiological and biochemical properties were all optimised for use with a carbapenem (Lomovskaya et al., 2017).

The potency of vaborbactam derives from its attachment to the serine side chain of β-lactamases from the boronate moiety (Hecker et al., 2015; Figure 2). Vaborbactam has the ability to extend the spectrum of carbapenems, such as meropenem, against gram negative bacteria that produce carbapenemases (Lee and Baker, 2018). It is potent against class A β-lactamases including GES and KPC in addition to class C β-lactamases. However, vaborbactam does not inhibit class B or class D carbapenemases (Lomovskaya et al., 2017). A combination of meropenem and the novel ß-lactamase inhibitor, vaborbactam, was produced to treat carbapenemase-producing gram-negative bacteria. Meropenem exhibits a broad-spectrum bactericidal action that is time-dependent, as with other β-lactams. Meropenem has historically been used as a last resort to treat gram-negative bacilli that produce β-lactamases. It binds to penicillin-binding proteins (PBPs) that prevents transpeptidation and consequently the formation of the cell wall (Jorgensen and Rybak, 2018).

Jorgensen and Rybak (2018) conducted a study with 2604 isolates of *P. aeruginosa* demonstrating an ineffective potentiation effect of vaborbactam with meropenem – likely due to other mechanisms such as intrinsic porin alterations and drug efflux or class B and D carbapenemases. The ability for *P. aeruginosa* to present with multiple mechanisms of resistance to carbapenems in the same strain was confirmed by de Oliveira Santos et al. (2018) when KPC-2's genome was sequenced. In addition, resistance to vaborbactam with meropenem is high among *P. aeruginosa,* demonstrated in 6/14 cases. However, the sample size of this study was too small to allow descriptive analysis (Jorgensen and Rybak, 2018).

Endogenous AmpC confers slight protection against imipenem in *P. aeruginosa*. Therefore, relebactam combined with imipenem was considered for KPC producing *P. aeruginosa* to potentially overcome *P. aeruginosa's* intrinsic resistance to imipenem, as previously described, and its ability to evade up-regulated efflux, unlike other BLIs. A dose of 4mg/L reduced the minimum inhibitory concentration of imipenem from 1-2mg/L to 0.25-0.5mg/L..
This demonstrates a significant augmentation of imipenem activity (Livermore et al., 2013), and a potential for future drug treatment for *P. aeruginosa.*

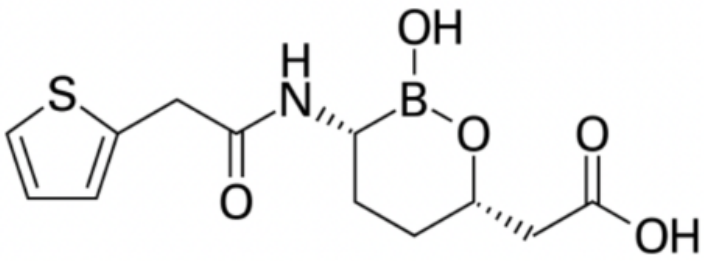

Figure 2: *The chemical structure of vaborbactam adapted from Hecker et al., (2015).*

Conclusion

Pseudomonas aeruginosa is an opportunistic gram-negative infection that has been identified by WHO to be a threatening infectious disease, especially among immunocompromised patients in hospitals. It has many intrinsic mechanisms of resistance and the ability to acquire further resistance. *P. aeruginosa* is gaining the ability, through KPC and other enzymes, to resist carbapenems, which underlines the demand for novel antibiotic treatments or enhanced treatments of current antibiotics. Novel β-lactamase inhibitors are being explored, but further controlled clinical trials are required to document the BLI combination with an antibiotic optimum for *P. aeruginosa* infections.

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VOLUME 3 (2018-2019)

UgSJ Undergraduate Science Journal University of Exeter

Anti-predator Responses of the Flat Periwinkle *Littorina obtusata* to water borne chemical cues: A comparison of different chemical stimuli

Ghalia Abel

Abstract

Anti-predator responses have developed in animals to increase their likelihood of survival. Behavioural responses can occur when exposed to predator chemical cues. The anti-predator responses of *Littorina obtusata* to predator (*Carcinus maenas* and *Nucella lapillus*), dead conspecific and dead heterospecific (*Patella vulgata*) stimuli were studied. Responses were measured by reaction time and standard distance moved, compared to a control (seawater). The results showed slower reactions when exposed to *C. maenas* stimuli. This could be an avoidance behaviour to evade predation. There was no relationship between reaction time and standard distance to other stimuli compared to the control. Reasons such as varying prey diet, predation pressures and the environment were discussed. These insights can help inform us of survival rates in animals.

Introduction

Animals have developed many different strategies to avoid predators and increase their likelihood of survival (Kats, 1998) Some animals, such as in the lubber grasshopper (*Romalea guttata*) secrete toxins to deter predators (Yosef & Whitman, 1992). Other animals use intimidation defence; for example, the peacock butterfly (*Inachis io*), hisses and exposes its eyespots to predators (Vallin, et al., 2005). Species also use behavioural anti-predator responses such as fleeing or avoiding predators (Dial, et al., 1989) (Stuart-Fox, et al., 2006) (Greisser, 2008), change colour (for example, the chameleon *Bradypodion transvaalense*) or produce an alarm call (in the case of many bird species) (Stuart-Fox, et al., 2006) (Greisser, 2008). However, anti-predator defences can be costly and species must assess the costs and fitness benefits of using them (Kats, 1998) (Endler, 1986). A mechanism in which the cost of anti-predator defence can be assessed includes the use of chemical cues from predators on prey (Dial, et al., 1989). These chemicals are released by predators and do not require physical predator-prey contact because they are air or water borne (Harvell, 1990).

Chemical cues for anti-predator defence may also be released by injured conspecifics and other species with similar predators (heterospecifics) (Wisenden, et al., 1999). These cues can be released as alarm signals to warn a species of an incoming predator (Luduc, et al., 2004). A study on the Crustacean *Gammarus minus* found that injured conspecific cues prolonged the time of first attack and survival (increasing fitness) (Wisenden, et al., 1999). Whilst, *G. minus* were attacked faster when exposed to injured heterospecific cues (decreasing fitness) (Wisenden, et al., 1999). In order to detect anti-predator cues, species must be able to detect and translate them using chemoreceptors (Kats, 1998) (Croll, 1983). In prosobranch gastropods the mantle tentacles, cephalic tentacles and tip of the siphon are major chemoreception organs (Croll, 1983). Hence, marine gastropods may be the ideal class for studying anti-predator responses to waterborne cues.

This study focuses on the behavioural responses of the prosobranch flat periwinkle (*Littorina obtusata)* to chemical cues from 2 natural predators (the European green crab (*Carcinus maenas)* and the dog whelk (*Nucella lapillus*)), a dead conspecific (*L. obtusata*) and a dead heterospecific (the common limpet (*Patella vulgata)* (Reimchen, 1982) (Largen, 1967). The responses measured were, the time taken for L. obtusata to react to the cues and the standard distance moved if a reaction occurred. Quick reactions and long distances were expected from predator and dead conspecific cues. However, little reaction from the dead heterospecific cue was predicted due to differences between predator-prey dynamics in different species. This experiment allows for further understanding into the fitness benefits of chemoreception within predator-prey dynamics.

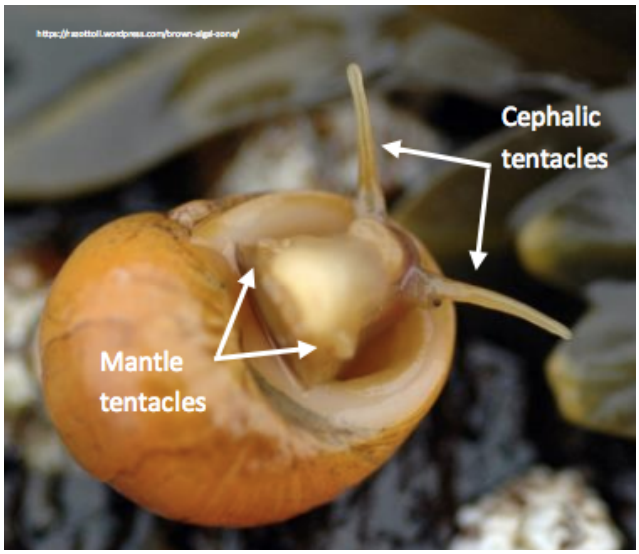

Figure 1. The ventral view of *Littorina obtusata* showing the mantle and cephalic tentacles.

Methods

Animal collection

Samples were collected on the intertidal rocky shore of Dale in Wales, UK during low tide. *L. obtusata* were collected every 2 meters (perpendicular to the sea) ensuring random sampling across the intertidal zone. A total of 50 *L. obtusata* were collected and kept in seawater. Then, 2 *C. maenas* and 3 *N. lapillus* were collected to test for predator stimuli responses. Numbers of predators collected ensured similar mass (of predator) to water ratio within chemical concentrations of predator stimuli. The predators were kept in separate trays with seawater. Additionally, one dead *L. obtusata* and one dead *P. vulgata* were collected to test for reaction to a dead conspecific and a dead heterospecific respectively.

Stimuli preparation

Stimuli were prepared in the laboratories of Dale Fort field studies centre. The stimulus species were each placed in separate 450 ml containers with 150 ml of seawater and left to stand for 30 minutes. This allowed for chemical cues of stimulus species to be released into the water. To an extent, water volumes used in this study followed a previous experiment by Duval et al. (1994) which studied the responses of Littoraria irrorata to water borne chemicals (Duval, et al., 1994). However, smaller quantities of seawater used for stimuli preparation in this study resulted from time constraints to ensure chemical concentration signals would be detectable to *L. obtusata*.

Procedure

To test the effects of stimuli on *L. obtusata*, 5 trays were prepared by drawing 4X4 cm grids using white board markers and then rinsed to remove excess ink that could harm the periwinkles. 1000 ml of seawater was added to each tray to ensure periwinkles were fully submerged in water. An individual periwinkle was inserted in the centre of a tray, covered and left to acclimatise for 5 minutes. Following acclimatisation, 3 ml of chemical stimuli was added directly onto the periwinkle to ensure contact and remove directional bias. The first movement of periwinkles was noted as the reaction time and was recorded in seconds. Movement of periwinkles was mimicked by hand using graph paper and following the gridlines on trays. Then, net distance was measured from start to end points using a ruler. Also, gross distance was measured by following the drawn route of periwinkles with string and measuring string length.

The Periwinkles were each measured in mm from the widest point at the base of the shell. Each stimulus experiment was repeated 24 times and seawater in trays was changed for every repeat. Furthermore, each periwinkle was left to rest for an hour before being put in a second trial.

Statistical Analysis

Two analyses of variance (ANOVA) were used to test for significance of stimuli trails to reaction times and standardised distance. They were followed by Tukey's Honest Significant Difference test which were run to confirm where the differences occurred between groups. Tests were run in RStudio 3.4.2 (Team, 2017). To ensure that size did not skew the distance moved, the distance and size measurements were standardised using this equation:

$$Standard\ Distance = \frac{\left(Net\ Distance / Gross\ Distance\right)}{Size}$$

Results

Periwinkle sizes ranged between 4-17 mm. The maximum net distance reached was 364 mm in response to the dead limpet stimuli and the maximum gross distance reached was 450 mm during control trials. The reaction time of *L. obtusata* was a significantly affected by stimuli trials (ANOVA, $F_{4,115}$= 5.336, P< 0.001). The reaction time was significantly slower when exposed to crab predator stimuli in comparison to the Control (Tukey HSD: P= 0.008) (Figure 2.). No other stimuli significantly affected the reaction time of *L. obtusata* in comparison to the Control (Tukey HSD: P > 0.05).

However, there was no significant relationship between *L. obtusata* standard distance moved and stimuli (ANOVA, $F_{4,115}$ = 2.062, P= 0.090) (Figure 3.).

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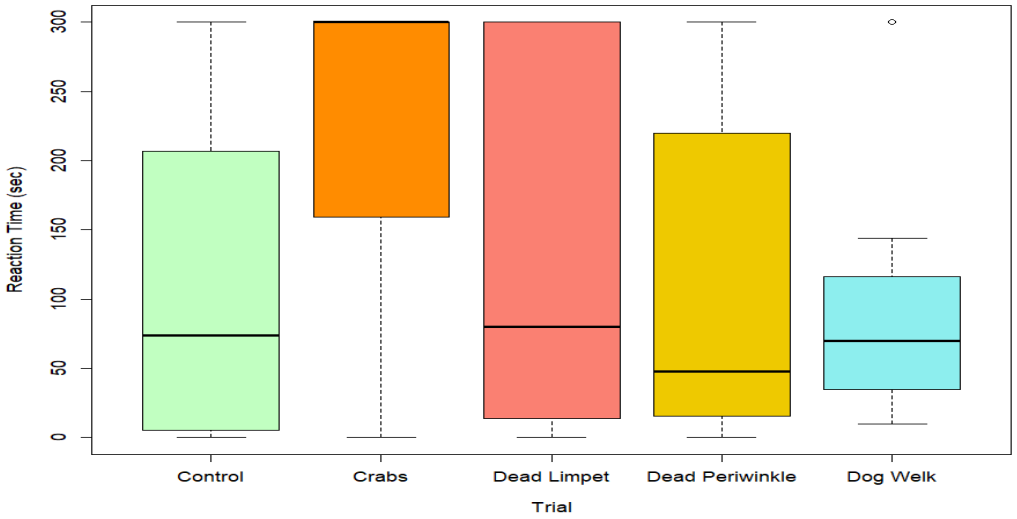

Figure 2. The Reaction Time (sec) of Periwinkles *Littorina obtusata* to different stimuli trials. The lines on the outside represent the range of reaction times. The coloured boxes represent the interquartile range and the solid line in the boxes is the median.

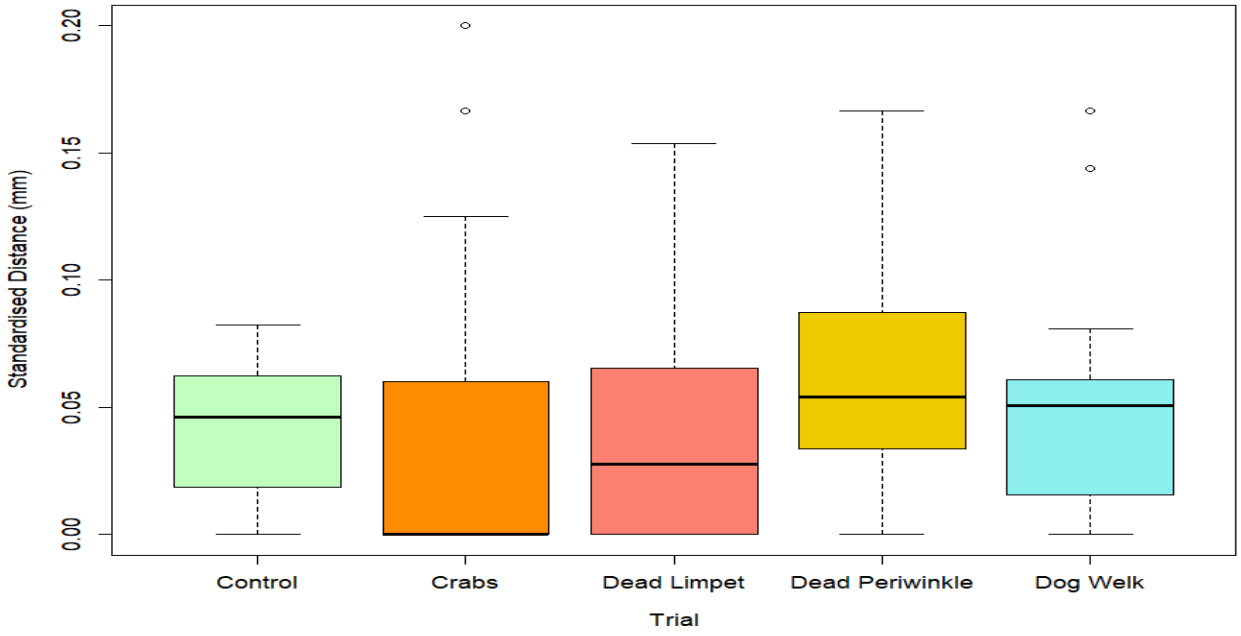

Figure 3. The Standard Distance (mm) of Periwinkles *Littorina obtusata* to different stimuli trials. The lines on the outside represent the range of reaction times. The coloured boxes represent the interquartile range and the solid line in the boxes is the median.

Discussion

It has been previously demonstrated in gastropods, that antipredator escape behaviour is caused by exposure to chemical cues from predators feeding on conspecifics and heterospecific snails (Rochette & Dill, 2000). However, the results of this experiment show a slow or no reaction when exposed to crab in comparison to control stimuli (Figure 2.). The reaction time could be due to the observed avoidance behaviour of hiding in the protective shell rather than escaping. This behaviour could be costly and decrease likelihood of survival, as *C. meanas* feed by cracking *L. obtusata* shells (Reimchen, 1982). However, in response to *C. meanas* predation cues, *L. obtusata* have developed phenotypic plasticity of their shell casing (Brookes & Rochette, 2007). This plasticity causes increased rates of calcification which in turn produces larger/thicker shells and protection against breakage (Reimchen, 1982) (Brookes & Rochette, 2007). Hence, avoidance may be a better option compared to escaping when exposed to predation by *C. meanas* and could increase the rate of *L. obtusata* survival.

The results also displayed quick reactions to dog whelk stimuli in comparison to crab stimuli but no significant difference compared to the control (Figure 3.). This could be an escape response of *L. obtusata* as it may be able to out-run a dog whelk. However, other factors may be taking place such as the varied diet of dog whelks (Largen, 1967). Prey respond to the metabolic by-products of predators (cues) which can be mediated by predator diet (Kats, 1998). For example, *Littorina littorea* had stronger reactions (escape) when exposed to predator cues of crabs fed on *L. littorea* in comparison to a fish diet (Jacobsen & Stabell, 1999). Dog whelks collected could have fed previously on different prey which may affect *L. obtusata* responses. In addition, *L. obtusata* reaction time was not affected by a dead conspecific or heterospecific stimuli (Figure 2.). This could be a result of predation pressure which can affect anti-predator responses (Kats, 1998). For example, the presence of dead conspecifics and heterospecifics may be a common occurrence in Dale rocky shore areas. Which may explain why *L. obtusata* moved rather than hid in the protective shell when exposed to the cues. Moreover, previous mark-recapture studies on *L. obtusata* found long shoreward movements of up to 15m when exposed to predator cues (Rochette & Dill, 2000). However, there was no relationship found between *L. obtusata's* standard distance moved and different stimuli in this experiment.

This could be an effect of exposure, as trays in this experiment did not contain any hiding or refuge areas such as rocks. The mark recapture study, however, was conducted in the field where refuge was possible (Rochette & Dill, 2000). Hence this lab study could be mimicking the effects of predator cues on gastropods in more exposed shore situations.

Future studies could simulate more controlled complex environments including rocks or seaweeds that act as shelter for *L. obtusata* to test whether escape behaviour is favoured over avoidance. Further studies could also look at the various colour morphs of *L. obtusata* in relation to the complex environments in order to understand the effects of camouflage on predator avoidance (Phifer-Rixey, et al., 2008).

In conclusion, *L. obtusata* reacted slowly and hid in response to crab stimuli and showed no change in reaction time when exposed to dog whelk, dead conspecific and heterospecific chemical cues. These responses could have been mediated by prey diet and predation pressure. Finally, the insights found can help us understand the adaptation of anti-predator responses in different environments and their relation to the fitness of species.

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CTX-M Type Extended Spectrum Beta-Lactamases: Acquisition, Action and Treatment

Alexandra Sebastiao

Abstract

CTX-M-type extended spectrum beta-lactamase (ESBL) *Enterobacteriaceae* are a rapidly evolving and spreading class of antibiotic resistant bacteria. *Klebsiella pneumoniae* and *Escherichia coli* in particular are the most prevalent and widespread strains carrying the *blaCTX-M* gene for beta-lactamase activity in the UK and Europe. Acquisition of *blaCTX-M* from *Kluyvera* has resulted in *Enterobacteriaceae* species that demonstrate resistance to large classes of beta-lactam antibiotics, including penicillins and third generation cephalosporins. Amino acid sequencing and trend observation has allowed predications to be made on the molecular mechanisms of action of CTX-M beta-lactamase enzymes. This could have useful clinical applications when deciding what antibiotics to administer for treatment of these infections. A substantial amount of research is being carried out to determine the most effective antibiotic treatments that minimize the power of selectors for these resistance genes.

Introduction

Since the discovery of Penicillin in 1928, antibiotics have played a vital role in the treatment of bacterial infections. However, overuse and misuse of antibiotics has led to resistance being acquired by numerous pathogens. Pathogenic resistance has become a serious problem worldwide and has had severe implications on the treatment of infectious diseases in recent years (Shaikh et al., 2015). Extended spectrum beta-lactamases (ESBLs) pose a critical threat among resistant bacteria. Beta-lactamase enzymes act on and cleave beta-lactam rings of extended spectrum beta-lactam antibiotics so they are no longer effective.

A major class of ESBL enzymes is CTX-M (DynaMed Plus, 2018), which is coded for by genes on plasmids most commonly found in *Escherichia coli* and *Klebsiella pneumoniae* (Livermore et al., 2006).

The first ESBL from the CTX-M group was isolated in Munich in the 1980s and was active on cefotaxime (Cantón et al., 2012). Over the past decade there has been a rapid rise in the prevalence of the CTX-M class of ESBL-producing bacteria globally, and it is increasingly becoming the most common type of ESBL (Paterson and Bonomo, 2005). Countries in Europe have conducted well-documented studies clearly showing this trend – the percentage of CTX-M ESBLs in Italy increased from 12.5% in 1999 to 38.2% in 2003; in Austria this percentage increased from 0% in 1998 to 85% in 2004 (Rossolini et al., 2008). This review focuses on the development, resistance mechanism, and treatment of CTX-M ESBL-producing *E. coli* and *K. pneumoniae* in Europe.

Methodology

A literature search was conducted using key terms such as "CTX-M", "ESBL-producing", "*E. coli* OR *Klebsiella pneumoniae*" to give results in the PubMed and TRIP databases. Due to the great variations in prevalence and epidemiology found between countries, searches became narrowed to Europe and the UK. Searches were then refined by use of truncation, more key words such as "extended spectrum beta-lactam*", and on the basis of location such as "UK OR Europe". A mixture of guidelines, reviews and primary research papers were collected to improve the reliability of the data. Papers were appraised using the Critical Appraisal Skills Programme checklist.

Results/Discussion

Evolution of blaCTX-M

CTX-M is the most common genetic variant of ESBL-producing bacteria (Shaikh et al., 2015). *blaCTX-M* encodes the CTX-M beta-lactamase enzymes which hydrolyse the beta-lactam ring of extended spectrum beta-lactam antibiotics, rendering them ineffective. This gene is present on plasmids within the resistant *E. coli* and *K. pneumoniae* bacteria. It is suggested that these species of *Enterobacteriaceae* evolved resistance through the conjugation and horizontal gene transfer with the *Kluyvera* species of chromosomal *bla* genes that are closely related to *blaCTX-M* (Shaikh et al., 2015). *Kluyvera* are freely present in the environment and are part of the normal human microbiome but occasionally cause opportunistic infections in immunocompromised patients (Cantón et al., 2012). Further evidence supporting this theory of resistance transfer are the similarities in the gene sequences adjacent to *blaCTX-M* of *Enterobacteriaceae* and the *Kluvyera* species (Cantón et al., 2012).

Mechanism of resistance

Beta-lactam antibiotics work by binding to a class of transpeptidases called penicillin binding proteins (PBPs), preventing them from forming cross-linkages between peptidoglycans (Bush and Bradford, 2016). Peptidoglycans are an essential component in bacterial cell walls. Cross-linkages between peptidoglycan chains cannot form without PBP enzymes, so the cell walls of bacteria become defective, weak, and unable to withstand the osmotic pressure within the cell. This results in cell lysis. The bactericidal effects of beta-lactams are further enhanced by triggering the release of autolytic enzymes, causing cell death. Beta-lactam antibiotics are therefore both bacteriostatic (by inhibition of cell wall synthesis) and bactericidal (by lysis and enzymatic destruction) (Williamson et al., 1986).

Based upon their amino acid sequences, CTX-M-type ESBL enzymes are classified as class A beta-lactamases according to the Ambler classification system (Hall & Barlow, 2005). Class A beta-lactamase enzymes hydrolyse serine (Hall and Barlow, 2005). They function by causing the beta-lactam substrate to enter its active site through an electrostatic attraction. The substrate is then better positioned in the active site through hydrogen bonding between the beta-lactam and the serine amino acid, forming an enzyme-substrate complex (Fisher et al., 2005). A series of chemical reactions then take place that results in the breaking of a bond in the beta-lactam ring. This molecular mechanism is demonstrated in Figure 1. The beta-lactam becomes inactivated through this hydrolysis and no longer displays its functional properties (Skagseth, 2012).

Treatment

ESBL-producing bacteria are highly resistant to penicillins, monobactams, and cephalosporins (DynaMed Plus, 2018). Most CTX-M ESBL-producing *E. coli* are resistant to third generation cephalosporins, including cefotaxime, ceftriaxone, cefixime, and ceftazidime (DynaMed Plus, 2018). Of the cephalosporin beta-lactams, CTX-M beta-lactamases hydrolyse cefotaxime very effectively, with minimum inhibitory concentrations (MICs) of 8-256µg/mL. In comparison, ceftazidime has a much lower MIC of 0.5-2 µg/mL (Eliopoulos and Bush, 2001) and many strains appear to be susceptible to ceftazidime (Livermore and Hawkey, 2005). Despite this evidence, UK guidelines strongly advise against their use for treatment of ESBLs because *in-vitro* susceptibilities do not always reflect clinical effectiveness (Hawkey et al., 2018).

CTX-M enzymes in particular have a high potency towards the penicillin 'pivmecillinam' and the monobactam 'aztreonam', which the guideline also strongly advises against for such infections (Hawkey et al., 2018). Resistance to non-beta-lactams is also possible amongst CTX-M ESBLs, including quinolones, and to a lesser extent, aminoglycosides like gentamicin (Hawkey et al., 2018).

Many CTX-M-producing *E. coli* also appear to be resistant to some beta-lactam/beta-lactamase inhibitor combinations like piperacillin-tazobactam (DynaMed Plus, 2018). In this case, the guideline instructs to confirm susceptibility before its use (Hawkey et al., 2018). Reasons the bacterium may not be susceptible *in-vivo* include: insufficient serum concentrations being achieved at adequate doses, resistance induction during treatment, or the inoculum effect (when a drug becomes less effective with a high bacterial load even though the bacteria was susceptible at low concentrations) (DynaMed Plus, 2018). Other combinations like ceftazidime-avibactam are strongly advised against, regardless of susceptibility testing results (Hawkey et al., 2018).

The preferred treatment for serious ESBL infections is carbapenem antibiotics (Hawkey et al., 2018). These include doripenem, meropenem, imipenem, and ertapenem. Ertapenem is a more narrow-spectrum carbapenem, so is generally favoured (Hawkey et al., 2018). Temocillin is a type of carboxypenicillin that can be effective against ESBL-producing *E. coli* and *Klebsiella*. Amoxicillin-clavulanate can be used against ESBL-producing bacteria if isolates have undergone susceptibility testing (Hawkey et al., 2018). Gentamicin can be administered to limit carbapenem use, however, *in-vitro* susceptibility must be confirmed, and attention given to other medications being taken due to high risk of nephro-toxicity and oto-toxicity with this drug (Hawkey et al., 2018). Antibiotics like colistin, polymyxin, tigecycline, temocillin and fosfomycin are used when treatment with carbapenems like meropenem or imipenem has failed or carbapenemase activity is suspected in ESBL-producing strains (Hawkey et al., 2018). These strains need immediate susceptibility testing to restrict the chances of carbapenem-resistant strains spreading (Hawkey et al., 2018). Table 1 shows the susceptibility of ESBL-producing *E. coli* of various CTX-M subtypes to several beta-lactam antibiotics. The evidence base used for treatments in this review were highly reliable NICE-accredited UK guidelines (Hawkey et al., 2018).

A primary research study in Austria investigated the prevalence of CTX-M ESBL-producing *Enterobacteriaceae* and their sensitivity to different antibiotics between 1998 and 2004. Laboratory polymerase chain reaction (PCR) techniques were used to isolate the *blaCTX-M* genes of samples taken. The results were interpreted using criteria set by the Clinical and Laboratory Standards Institute (NCCLS, 2004), supporting their reliability. Of the 149 ESBL-producing *Enterobacteriaceae* samples collected, 38 *E. coli,* and 11 *Klebsiella* isolates were producers of CTX-M. The antibiotic sensitivity results are summarized in Table 2. In general, there is a higher prevalence of *E. coli* compared to *Klebsiella* (Eisner et al., 2006). CTX-M-producing *E. coli* strains also tend to be more susceptible to antibiotics, showing higher susceptibility to amikacin, gentamicin, and piperacillin-tazobactam compared to *Klebsiella* isolates (Eisner et al., 2006). *Klebsiella* showed higher comparable susceptibility to ciprofloxacin and both isolates were 100% susceptible to meropenem (MIC - ≤0.25 mg/litre). A larger pool of data and testing would be needed to support the results of the study, which only included 149 isolates. Furthermore, to be certain the results are clinically applicable, more studies comparing the effectiveness of antibiotics in patients infected with CTX-M ESBL-producing *E. coli* and *Klebsiella* are needed (Eisner et al., 2006).

Conclusion

Antibiotic resistance is a critical issue worldwide. The increasing prevalence and spreading resistance of CTX-M type ESBLs has become a global problem and poses challenges in the treatment of these bacteria. It is becoming increasingly more difficult to pinpoint and attribute antibiotic resistance of bacteria to one particular gene and enzyme, highlighting the need to be conscious of multiple resistance genes. This has clinical relevance when treating patients for infections with these bacteria.

Accurate and efficient identification of these resistance mechanisms would aid in the discovery and design of new antibiotics. Some antibiotic treatment studies had insufficient data comparing their effectiveness with other antibiotics to determine whether they could be used against ESBL-producing *E. coli* and *K. pneumoniae.* Furthermore, there are few clinical trials comparing the effectiveness of carbapenems to other treatments, which could be a significant area of comparative research to explore in the future, focusing on the expanding CTX-M-type ESBLs.

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Figures and Tables

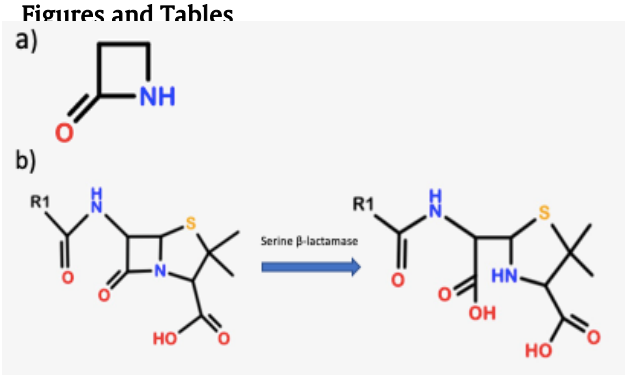

Figure 1. a) Chemical structure of a beta-lactam ring b) Action of beta-lactamase on beta-lactam ring of an antibiotic. Beta-lactam ring is broken so antibiotic is no longer effective Image adapted from (Skagseth, 2012)

Table 3
Susceptibility to various beta-lactams of *E. coli* producing CTX-M derivatives.
*beta-lactam susceptibility of *E. coli* without beta-lactamase activity shown for comparison
(Rossolini et al., 2008)

	MIC of antibiotic (Mg/L)			
enzyme	cefotaxime	ceftazidime	cefepime	aztreonam
None*	<0.06	<0.06	<0.06	0.06
CTX-M-3	>256	32	128	128
CTX-M-15	>256	256	64	64
CTX-M-54	8	128	1	2
CTX-M-1	>128	6	48	48
CTX-M-32	>128	>256	64	>256
CTX-M-9	16	1	No data	4
CTX-M-16	16	8	No data	4
CTX-M-18	64	2	16	64
CTX-M-19	4	128	4	4

Table 2. Antibiotic susceptibility rates of Klebsiella and E. coli isolates carrying blaCTX-M in a primary research study conducted in Austria between 1998 and 2004 (Eisner et al.,

Antibiotic	Susceptible blaCTX-M +ve *E. coli*	Susceptible blaCTX-M +ve *Klebsiella*
Meropenem	100%	100%
Amikacin	100%	18%
Gentamicin	63%	36%
Cotrimoxazole	66%	55%
Ciprofloxacin	45%	73%
Piperacillin-tazobactam	92%	54%

Acknowledgments

The Editing Team would like to recognise the invaluable contributions of the college staff and team of peer reviewers whom, without their time and effort, the publication of this volume would not have been possible.

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This Journal was funded by the generous donations of the Alumni Annual Fund and printed by the Student Guild Print Room.

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