Prevalence of *Staphylococcus aureus* in denture plaque in a group of denture wearing in-patients

Formatted: Justified

1. GENERAL INTRODUCTION

Formatted: Centered

Formatted: All caps

Formatted: Justified

Since Sir Alexander Flemming discovered the *Penicillium* mould growing on a piece of bread, mankind has been involved in what one may call an evolutionary arms race between Humans and Bacteria. In 1941 penicillin became a mainstay of treatment for acute bacterial infection and by 1946 antibiotic-resistant bacterial strains began to emerge. The "Hospital *Staphylococcus*," was a strain of *Staphylococcus aureus* which was resistant to penicillin, erythromycin and tetracycline among other antibioitcs. The subsequent development of methicillin and flucloxacillin helped to bring these bacteria back under control by providing a therapeutic alternative, but in the early 1960s the first clinical case of methicillin-resistant *Staphylococcus aureus* (MRSA) infection was reported in the United Kingdom. Since its discovery reports of MRSA have escalated throughout the world with infections being reported in Alaska, Hawaii, USA and India (*Source: Center For Disease Control (CDC) Atlanta MMWR June 2006).*

S. aureus can be found in the anterior nares of 26.7% of the elderly population (Grundmann et al 2002) without causing clinical signs and symptoms of infection. The nature of MRSA colonization is no different, an individual may be a carrier of the bacterium but not develop any symptoms. This poses a difficult challenge to hospital units as every individual entering or leaving the institution

is a possible source or carrier of this bacterium. The Centre for Disease Control (USA) has recently published a bulletin highlighting the global nature of MRSA infection control, citing the infection of two hospitals in Canada with MRSA potentially introduced by a patient from the Punjab region of India. MRSA has since become endemic in the first contact institution in Vancouver and is beginning to spread to another healthcare institution in Winnipeg, Canada.

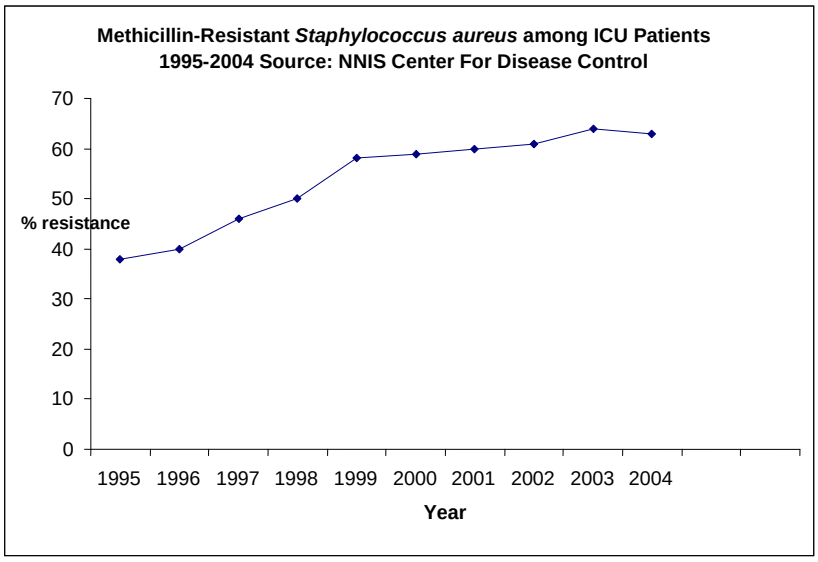


Fig1.1 MRSA positive patients among ICU patients in the USA

Antibiotic resistance, shown by bacteria, can be conferred in several ways. Resistance can occur as a consequence of natural properties of the bacterium, which is referred to as intrinsic resistance. An example of intrinsic resistance would be metronidazole resistance in aerobic bacteria; these bacteria are not susceptible to the action of this antibiotic as they do not have an anaerobic internal cellular environment which is required to activate this drug. The other

Comment [j1]: Name figs and tables after chapter helps finding them when discussing

Formatted: Font: Not Italic

Formatted: Font: Bold

Formatted: Font: Not Italic

Formatted: Justified, Indent: First line: 0"

Formatted: Justified

Formatted: Justified, Indent: First line: 0"

method of resistance is acquired resistance in which a previously susceptible population becomes resistant to the antibiotic after exposure to this agent. This may be via selection through mutation or acquisition of antibiotic-resistant genes. Some of these evolutionary changes have been observed by microbiologists and a brief description of each is as follows:

- **Changes in cell wall permeability**

This phenomenon appears to be more common in Gram-negative bacteria. Altering the cell wall constituents can lead to decreased permeability to the antimicrobial thereby reducing its efficacy if its target site is within the bacterial cell. Antimicrobials which require active transport across the cell wall can have their effects diminished by changes to cell wall transfer proteins and reduction in the number of binding sites (Jarlier, Gutmann and Nikaido 1991).

- **Anti microbial inactivation**

Some bacteria have evolved to produce extra cellular enzymes which will break down the active elements of anti microbials, reducing their efficacy. The most commonly seen example of this is β-lactamase which is released by Gram-negative bacteria; it breaks down the β-lactam ring present in antibiotics such as penicillin and in cephalosporins. The integrity of the β-lactam ring is necessary for the anti-microbial to inhibit peptidoglycan synthesis (Harvey and Hunter 1999).

synthesis.

- **Alteration of target site**

Formatted: Justified

Comment [N2]: REFERENCE!!

Formatted: Bullets and Numbering

1

Target site alteration is a mechanism of bacterial resistance employed by numerous bacteria against a wide range of antimicrobials. Alterations can range from a single unique mutation to the incorporation of extra-cellular DNA resulting in major cell alterations. The modification of penicillin binding proteins is utilised by *Neisseria meningitidis, Enterococcus fecium* and by MRSA.

In MRSA it is the presence of the SCCmec chromosome which allows the expression of the mecA gene complex. This codes for production of an abnormal penicillin binding protein called PBP2a (Fraimow and Abrutyn 1995). This protein has a reduced binding affinity for β lactam antibiotics (Guignard, Entenza and Moreillon 2005) thereby conferring resistance to this group of antibiotics.

- **Alteration of metabolic pathway**

Formatted: Bullets and Numbering

This is one of the most complex methods of bacterial resistance; . Tthe development of alternative metabolic pathways, which effectively by-pass the need for certain enzymes or proteins altogether. For example some sulfonamide-resistant bacteria such as Streptococcus *pyogenes* do not need the precursor para-aminobenzoic acid (PABA) in nucleic acid synthesis. Since the PABA is inhibited by the presence of the sulfonamide the bacteria have adapted to metabolise preformed folic acid.

The frequency of antimicrobial-resistant pathogens in the UK is of increasing concern to both medical and dental practitioners. The mouth hosts an inordinately large number of diverse microbes. More than 700 taxa* of which 50% are yet to be cultivated have been detected in the oral cavity (Jorn, Bruce et al 2005). ~~Of these the antibiotic-resistant pathogen Methicillin-Resistant~~

~~1~~

~~*Staphylococcus aureus* (MRSA) is responsible for widespread infections of hospital bound patients in the UK and abroad. Wylie et al (2006) reported a death rate of 34% within 30 days amongst patients infected with MRSA whilst Das, O'Connell and Lambert (2006) stated that S. *aureus* infections mostly affect the~~

Formatted: Justified

** ("taxa": a term used to include cultured and uncultured bacteria, as opposed to "species": a term which only includes cultured bacteria)*

Of these the antibiotic-resistant pathogen Methicillin Resistant *Staphylococcus aureus* (MRSA) is responsible for widespread infections of hospital bound patients in the UK and abroad.

Wylie et al (2006) reported a death rate of 34% within 30 days amongst patients infected with MRSA whilst Das, O'Connell and Lambert (2006) stated that S. *aureus* infections mostly affect the elderly and emphasized the need for an improved strategy in the control and management of these infections.

The problem of MRSA was a key factor in the recent NHS "Clean Hands Campaign." This resulted in the introduction of hand washing protocols and the availability of alcohol hand gels beside hospital beds in all NHS trusts throughout the country.

Evidence that MRSA is present in the oral cavity and may also be present on removable oral prostheses is increasing. Current demographics

suggest that in the UK over 9 million people wear an oral prosthesis (Adult Dental Health Survey 1998). If the MRSA pathogen is able to survive and replicate on oral prostheses then NHS trusts and hospitals may need to review their current eradication and management protocols.

2. Literature Review

Formatted: Font: Bold

Formatted: Justified

2.1 Staphylococci

Staphylococcus aureus is a Gram-positive coccus which appears as grape like clusters when viewed under a microscope. It has large, circular yellow colonies, frequently [exhibiting] β-haemolysis when cultured on blood agar (Ryan and Ray 2004).

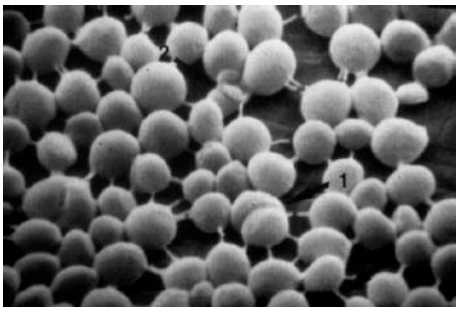


Fig 2.1 An SEM to show colonies of Staphylococci (Rollins and Joseph 2000)

Formatted: Font: 10 pt, Not Italic
Formatted: Font: 10 pt
Formatted: Centered, Indent: First line: 0"
Formatted: Justified
Formatted: Justified, Indent: First line: 0"

Staphylococci are considered by some microbiologists to be one of the most versatile and successful of all pathogenic bacterial genera (Cassell 1982). Some species of staphylococci are present as commensal organisms whereas others maintain the ability to cause invasive and life threatening disease. As opportunistic pathogens they are most frequently found causing disease in immuno-compromised patients.

Formatted: Justified

S. aureus is associated with a range of oral infections including; angular cheilitis, infected root canals, facial cellulitis, osteomyleitis of the jaws, parotitis, stomatitis, gingivitis and dentoalveolar abscesses (Cassell 1982, Smith, Jackson and Bagg 2001).

2.1.2 Staphylococci in Humans

Formatted: Justified, Indent: First line: 0"
Formatted: Justified

~~1~~

Staphylococci have been isolated from supragingival plaque (Theilade et al 1982) and from subgingival plaque (Kamma et al 1999) in healthy individuals. In these studies different species of staphylococci were detected. One study showed that upto 84% of 1-5 year olds attending a paedodontic department carried staphylococcal species. Of these 33% were *S. aureus* and 5% were MRSA (Miyake et al 1991). In 2000Jackson and Bagg ~~in 2000~~ found that up to 64% of healthy children carry *S. aureus* in the oral cavity.

By the first 10 days of life 90% of infants are colonized with *S. aureus*, the carrier rate in the normal population rarely falls below 20% (Cassell 1982). The presence of *S. aureus* in the absence of clinical signs and symptoms of infection indicates colonisation with this microorganism rather than infection and therefore does not always involve treatment. It can survive on domesticated animals and on dry surfaces for several hours. *S. aureus* can also host bacteriophages which harbour virulence determinants such as Panton-Valentine leukocidin which will increases the virulence and pathogenicity of this bacterium (Emerging Infectious Disease Dispatch CDC Atlanta 2004).

S. aureus can infect skin tissues when the surface has been damaged. It can cause furuncles, carbuncles, acne and impetigo. In infants S.*aureus* can release an exfoliative toxin which can cause Scalded Skin Syndrome. This may persist for upto 7 days resulting in a 20% mortality rate (Curran and Al-Salihi 1980, Cassell 1982). *S.aureus* infections can be spread through contact with pus from an infected wound, skin to skin contact and contact with everyday objects such as sheets, towels, clothing or athletic equipment.

Formatted: Justified, Indent: First line: 0"

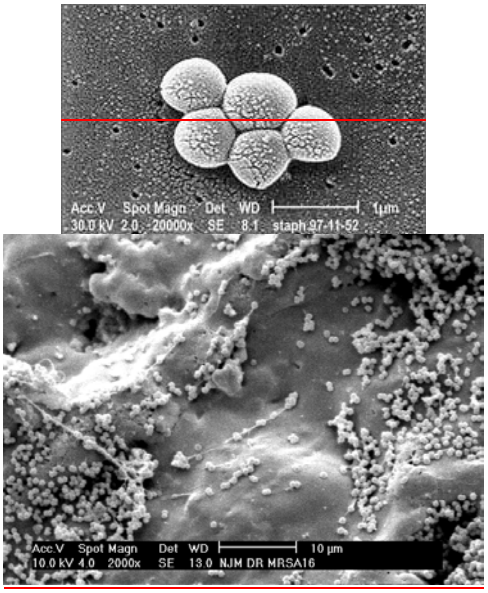
Formatted: Justified

2.1.3 Oral carriage of Staphylococci

Studies of adult populations have shown that between 94-100% are carriers of *Staphylococcus* sp (Percival, Challacombe and Marsh 1991) with oral carriage of *S.aureus* ranging from 24-36% (Jackson & Bagg 2001). Theilade and Budtz-Jorgensen, in 1988, hypothesised that the presence of prosthetic devices in the oral cavity, such as acrylic resin dentures, may encourage the carriage of staphylococci. In 1996 Tawara, Honma and Naito showed that to reduce MRSA in the oral cavity both antibacterial rinsing of the mouth coupled with stringent denture cleansing need to be employed.

2.2 Methicillin-Resistant *Staphylococcus aureus* (MRSA)

MRSA is a specific strain of the bacterium *Staphylococcus aureus* which has developed antibiotic resistance to all penicillins, methicillin and other narrow spectrum β-lactamase-resistant penicillin antibiotics (Foster 1996).



1

Fig2.2 A SEM showing ~~a small grouping of MRSA CDC© Jim Biddle~~colonies of MRSA on acrylic resin (Ready 2006)

Formatted: Font: (Default) Arial, Not Italic

Formatted: Font: Not Italic

Formatted: Justified

In the UK the number of deaths due to MRSA infection has been estimated to be approximately 3000 per year (Johnson, Pearson and Duckworth 2005). *Staphylococcus aureus* accounts for up to half of all UK hospital infections. In 2004 Cooper, Medley, Stone et al made the disturbing discovery that the MRSA bacterium can replicate and exist inside *Acanthamoeba polyphaga*. This is a single celled amoeba which engulfs and digests environmental bacteria as a food source including pathogens such as MRSA. However, instead of being destroyed with the cell MRSA is able to survive and replicate inside the amoeba increasing numbers by 1000 fold. *Acanthamoeba* spp. can spread by airborne currents which may further increase the infective potential of MRSA. Currently more research into this area is being conducted by the University of Bath in the department of Pharmacy and Pharmacology and at the UCL Eastman Dental Institute.

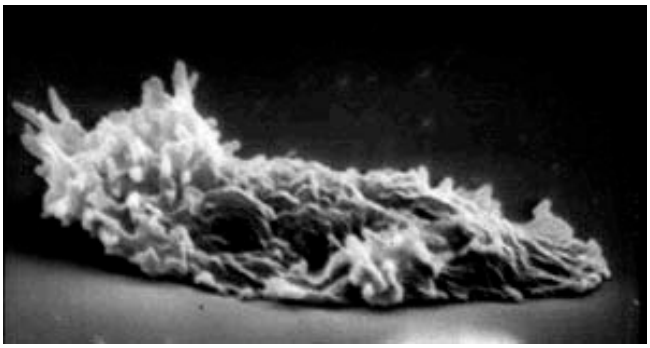


Fig 2.3 S.E.M. of Acanthamoeba castellanii Image courtesy of Dr Steven Dobberstei, University of Edinburgh

Formatted: Font: Not Italic

Formatted: Font: Not Italic

Formatted: Font: Not Italic

1

Formatted: Justified

2.2.1 Clinical Symptoms of MRSA Infection

Patients from whom MRSA is identified tend to be colonised with the pathogen as opposed to being infected. Colonisation refers to the presence of the organism in the nose, skin or back of the throat, but without showing any symptoms of illness. However, immune-compromised and other high risk groups, can succumb very quickly to MRSA infection. A prospective study carried out by Das, O'Connell and Lambert in 2006 found that the mortality rate in MRSA infected groups at a UK University Hospital was 33%. They found that the most common foci for MRSA infection was an intra-vascular device which accounted for 33% of MRSA infections.

MRSA symptoms vary dependent on the part of the body which has been infected. MRSA often appears as a skin infection resembling abscesses, these can spread causing infection in the urinary tract, or the bloodstream. As mentioned earlier, Wylie et al report a death rate of 34% within 30 days among MRSA infected patients.

MRSA infection is thought to be mainly nosocomial (infection due to being hospitalised) occurring predominantly in the elderly and the immuno-compromised (Maudsley at al 2004). However, it has also become widespread in nursing homes, with one study showing a prevalence of up to 17% of residents testing positive from cultures of the nose or fingers (Fraise, Mitchell, O'Brien et al 1996). It is thought that many of these infections or colonisations are due to previous hospital admissions of the occupants in the preceding year.

2.2.2 MRSA in the United Kingdom

In the United Kingdom two strains of MRSA have emerged as the most prevalent nosocomial pathogens these are EMRSA 15 & EMRSA 16 (Johnson et al 2005). The “E” prefix signifies that these strains may have epidemic potential. Most recently a new strain, classified as EMRSA 17 that is resistant to multiple antibiotics has been identified in the UK (Aucken et al 2002). EMRSA 15 was initially identified in the south-east of England in 1991, quickly spreading to hospitals around England. EMRSA 16 originated in Kettering (Stone 1997) around the same time as EMRSA 15, where it caused 400 infections and was responsible for 7 attributable deaths (Cox et al 1995).

2.2.3 Community acquired MRSA

Community-acquired MRSA (CA-MRSA) is distinct from the EMRSA variants as it is known to cause infections in healthy individuals with no previous exposure to healthcare environments (Press release; Center for Disease Control, USA 2005).

Symptoms range from minor skin and soft tissue inflammation to necrotizing pneumonia which quickly destroys lung tissue due to the ability of some strains of CA-MRSA to release a cytotoxin called Panton-Valentine leukocidin which attacks the patient’s own leukocytes (Yamamoto et al 2005).

A recent study commissioned by the European Society of Clinical Microbiology and Infectious Diseases in 2007 found that of 2,433 *S. aureus* strains isolated at the Institute of Hygiene, Graz Austria, 20.5% of the positive

MRSA (mecA-gene present) samples were CA-MRSA. This is a much higher number then anticipated and further supports the need for similar studies in different institutions and countries.

2.2.3 Oral Significance of MRSA

Recent studies have suggested that MRSA colonisation of the oral cavity is more common then previously thought. One study found that 5% of oral specimens containing S.*aureus* were methicillin-resistant strains (Smith, Robertson, Tang et al 2003). Carriage rates of 10% on denture surfaces of denture wearing patients visiting a clinic in Tokyo have been reported by Tawara, Honma and Naito in 1996. Another study showed the prevalence of MRSA as high as 19% from the mouths of an elderly institutionalised group (Owen 1994). The tongue appears to be the most common area for MRSA isolates with patients reporting erythema, swelling, pain and a burning sensation from the mucosa.

The presence of MRSA in the oral cavity poses great cross infection risks for dentists and patients alike. To date, there are two documented cases of MRSA transmission in a dental setting. These are reported to be from a general dental practitioner to patients (Martin and Hardy 1991). It is thought that the dentist was colonized after being a patient in hospital.

2.2.4 MRSA Treatment

Frequent attempts have been made to eradicate MRSA from patients and medical staff. Vancomycin and teicoplanin are the current antibiotics to which MRSA is susceptible. Both of these drugs have a low oral absorption and must therefore be administered intravenously for systemic infections. The discovery of several strains of MRSA which do not respond to vancomycin treatment is causing great concern. These have been termed vancomycin intermediate resistant *Staphylococcus aureus* or VISA (Schito 2006). Currently there are few alternatives on the horizon to treat MRSA infections. Platensimycin, a new class of antibiotic which has been demonstrated to successfully combat MRSA infection is currently being developed by Merck Pharmaceuticals (Nature 2006).

2.2.5 MRSA Infection of Denture Surfaces

Clinical evidence shows that oropharyngeal carriage of MRSA can be difficult to eradicate (Smith, Jackson and Bagg 2001). Eradication of throat carriage of MRSA has been achieved by using 0.2% chlorhexidine coupled with nasal mupirocin (an antibiotic effective against MRSA) and chlorhexidine body washes (Balfour, Higgins, Brown et al 1997).

It has been hypothesized that MRSA may preferentially colonise denture/acrylic resin surfaces and act as a source of cross infection (Rossi et al 1997). Calcium in the saliva acts as a bridge between the acrylic resin beads and the negatively charged surfaces of the bacteria. A Japanese study has shown that 10% of denture wearers are carrying MRSA on the surface of their dentures (Tawara, Honma and Naito 1996). Lewis at al (2006) found that of 100 patients attending for routine prosthetic appointments at the Eastman dental

hospital, 25% harboured Methicillin sensitive *S. aureus* on their dentures, with 1% testing positive for MRSA.

This raises several problems for geriatric institutionalised denture wearers where the denture cleaning is often carried out by care workers, introducing the possibility of horizontal transmission. In a recent study, eradication of MRSA from denture surfaces has proved to be difficult. Daily cleansing for 2 weeks using an over the counter denture cleaning product has shown to have little effect on MRSA levels (Tawara, Honma and Naito 1996). Rossi et al (1997) found that only heat sterilisation or re-making the dentures were the only ways to totally eradicate MRSA colonization from oral prostheses.

2.2.6 Hospital Screening MRSA Protocols

Formatted: Font: Bold

Investigation by the present author has revealed that current MRSA screening protocols in place at major London Hospitals, do not to include microbiological analysis of denture plaque. At UCLH, pre-surgical protocols do ask for swabs to be taken of the nose and any wounds, but no oral swab is currently taken (Infection control services UCLH). In addition, current treatment protocols for the eradication of MRSA from patients do not include any disinfection treatment of dentures. This may increase the possibility of the denture acting as a reservoir for MRSA which may explain the difficulty some hospitals are having in removing persistent MRSA infection from their wards.

1