METHICILLIN RESISTANT *S. AUREUS* IN AUTOPSY CASES

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Abstract

Objective To determine whether hospital stay predisposes to nasal colonisation with *Staphylococcus aureus* and Methicillin Resistant *S. aureus* (MRSA).

Method Nasal swabs were taken from cadavers undergoing post-mortem examinations at the mortuary of St. Luke's Hospital. The swabs were taken to the Bacteriology Laboratory where attempts were made to culture *S. aureus*. Vitek® Gram Positive Susceptibility Cards were used for antibiotic susceptibility. MRSA positive organisms were tested using Penicillin Binding Protein Latex Agglutination.

Key Findings Ninety-three swabs were taken. The proportion of *S. aureus* nasal carriage was similar in both hospitalised and non-hospitalised groups. However, 8 out of 15 (53%) *S. aureus* carriers in the hospitalised group were MRSA positive, compared to 4 out of 19 (21%) *S. aureus* carriers in the non-hospitalised group.

Conclusion Hospitalisation increases the incidence of MRSA carriage compared to the non-hospitalised population.

Key words MRSA, *Staphylococcus aureus*, nasal carriage, autopsy

Introduction

Staphylococcus aureus is an important cause of human disease. Although it is most often associated with skin and soft tissue infections, it has numerous manifestations including conditions with low morbidity and mortality, such as folliculitis and food poisoning, and others which cause fatal systemic illnesses, such as endocarditis and toxic shock syndrome.[1]

S. aureus colonises between 30 to 50 percent of the healthy adult population.[2] The anterior nares are the most consistent site of colonisation.1 Although the bacteria are normally harmless, they can cause serious infections when the opportunity arises.[3] *S. aureus* can develop resistance to a wide variety of antibiotics. Methicillin resistance confers resistance to all penicillinase-resistant penicillins and cephalosoporins.[2] MRSA infections have been associated with increased morbidity and mortality and hospital costs.[4]

Nasal carriage of *S. aureus* has become a means of persistence and spread of multiresistant Staphylococci, especially MRSA. Because MRSA can resist practically all types of antibiotics, they have become a public health threat, in the context of hospital-acquired infections and more recently as community-acquired diseases.[5] Factors associated with MRSA colonisation include prior antibiotic exposure, particularly incomplete or repeated courses of antibiotics, prolonged hospitalisation, surgery, admission to an intensive care unit, living in a nursing home, and close proximity to a patient colonised or infected with MRSA.[6]

The aim of the study was to determine whether hospital stay predisposes to nasal colonisation with *S. aureus* and MRSA by comparing two cohorts, one which was hospitalised and one that had not been admitted to hospital within the previous six months.

Method

Nasal swabs were taken from cadavers undergoing post-mortem examinations. These were divided into 2 categories, those that were hospitalised for at least 24 hours and those that were not hospitalised in the previous 6 months. Individuals who had drowned or who had severe facial injuries were excluded from the study. Approval from the Faculty and University Research Ethics Committee was obtained to carry out this project.

The nasal swabs were taken to the Bacteriology Laboratory at St. Luke's Hospital (SLH) where attempts were made to culture *S. aureus*. In this way *S. aureus* nasal carriers were identified. The nasal swab was first cultured on Mannitol Salt Agar (MSA), which is a selective medium for the isolation of Staphylococcus. spp. Most other bacteria are inhibited by the high salt concentration. Yellow colonies from the MSA were sub-cultured on blood agar, nutrient agar and DNAse agar. Catalase, coagulase and DNAse tests were then performed. *S. aureus* is catalase, coagulase and DNAse positive. A Gram-stain was also done to verify that the organisms were Gram positive cocci in clusters.

S. aureus organisms were further tested for their antibiotic susceptibility using Vitek® Gram Positive Susceptibility Cards which indicates the range of antibiotics that the organism is sensitive or resistant to. MRSA positive organisms were tested using Penicillin Binding Protein (PBP) Latex Agglutination, which is a confirmatory test for MRSA, since it detects the mutant enzyme PBP2a.

Results

A total of 93 swabs were taken from cadavers undergoing post-mortem examination during the period of study (12 months). The number of cases studied is heavily weighted in favour of the male sex. There were approximately equal numbers of cadavers in each age group with a mean age of 55 years (range 13 to 93 years). Out of the 65 males, 24 were hospitalised and 41 were not hospitalised. From the 28 females, 18 were hospitalised and 10 were not hospitalised.

Figure 1 shows the incidence of *S. aureus* in the nasal swabs studied in both hospitalised and non-hospitalised cases. The proportion of *S. aureus* nasal carriage was similar in both groups, with 36% of the hospitalised population having *S. aureus* nasal carriage compared to 37% of the non-hospitalised population. The incidence of *S. aureus* colonisation appears to be greatest in the 41 to 65 age group with 54% of the cadavers in that age group having *S. aureus* colonisation.

The picture changes radically when one looks at the incidence of Methicillin Resistant *S. aureus* (MRSA) carriage and Methicillin Sensitive *S. aureus* (MSSA) carriage. There are striking differences between the hospitalised and the non-hospitalised group. Eight out of the 15 *S. aureus* carriers (53%) in the hospitalised group, compared to 4 out of the 19 *S. aureus* carriers (21%) in the non-hospitalised group were MRSA positive, with a p value of 0.0505.

There were gender and age differences. MRSA colonisation appears to be relatively more frequent in females, since 5 out of 9 females had MRSA, when compared to 7 out of 25 males. None of the cadavers under 40 years of age had MRSA.

Many of the *S. aureus* nasal carriers exhibited resistance to a range of antibiotics (Figure 2). The resistance was much more common in the hospitalised cases, indicating that MRSA strains are more aggressive in the hospital setting. Most *S. aureus* strains were resistant to penicillin. All the hospitalised cases that were MRSA positive were also resistant to ofloxacin, whereas 2 out of the 4 cases of MRSA nasal carriage that were not hospitalised before death were resistant to ofloxacin. This shows a correlation between methicillin/ oxacillin resistance and ofloxacin resistance.

Most of the MRSA cases were also resistant to erythromycin with 6 out of the 8 MRSA positive nasal carriers that were hospitalised were erythromycin resistant. Two out of the 4 MRSA cases that were not hospitalised were also resistant to erythromycin. There were 2 cases (1 from the hospitalised group and 1 from the non-hospitalised group) which were resistant to erythromycin, but were not methicillin resistant.

Some of the cadavers had *S. aureus* strains which showed intermediate resistance to some antibiotics, namely fusidic acid (4 cases), fosfomycin (3 cases), rifampicin and erythromycin (2 cases each).

Discussion

These results show that hospitalisation increases the incidence of MRSA carriage compared to the non-hospitalised population. Although the incidence of MRSA carriage in the hospitalised group was more than twice the incidence in the non-hospitalised group, a p value of 0.0505 was obtained since a limitation of the study was the small number of cases studied.

The incidence of MRSA carriage in the non-hospitalised cases in this study is similar to that found by Dall' Antonia et al[7] who report an 8% incidence of MRSA in patients on admission to a United Kingdom healthcare institution. However other studies give a wide range of values; an extensive study carried out in the United States showed an incidence of MRSA of only 0.8%1 while another study in Lahore (Pakistan) showed that MRSA colonisation was found in 2.89% of the population.8 Skov9 has shown

that in the Nordic countries (Denmark, Finland, Iceland, Norway and Sweden) the incidence of MRSA infections was successfully kept at low levels (<1%). This is attainable by the conservative use of antimicrobial consumption, prescribing of narrow-spectrum antibiotics, screening patients, treating MRSA positive patients in isolation and the prevention of transmission through appropriate hand hygiene.[10] When preventive measures are strictly enforced, it is possible to keep the incidence of MRSA extremely low.

Between 2008 and 2009, Maltese hospitals reported a 55% incidence of MRSA from invasive S. aureus isolates.[11] This correlates well with the results in this study which have shown that more than half of the S. aureus carriers were methicillin resistant amongst the hospitalised group.

The widespread use of antibiotics in hospitals is universally acknowledged as the critical factor for the development of antimicrobial resistance. It was shown that the level of consumption of broad spectrum penicillins, especially those containing a beta-lactamase inhibitor, cephalosporins, in particular second generation cephalosporins, and macrolides at SLH was significantly greater than the median obtained from a pan-European study entitled 'Development of Strategies for Control and Prevention of Antibiotic Resistance in European Hospitals'.[12]

Until recently, most MRSA cases were found in the nosocomial setting. However, community-acquired MRSA continues to evolve and has been associated with both colonisation and infection. Strains of community-acquired MRSA are normally more sensitive to other antibiotics than hospital-acquired MRSA strains.[13] The incidence of MRSA in the community is also on the increase due to the widespread and overuse of antibiotics. Since nasal carriage triples the chance of developing bacteraemia with *S. aureus*,[3] the incidence of MRSA nasal carriage should not be underestimated.

Conclusion

The study shows that hospital stay does not increase *S. aureus* nasal colonisation when compared to the non-hospitalised group. However, the incidence of MRSA was much higher in the hospitalised group. The study also shows a relatively high incidence of MRSA cases in the community. The prevalence of MRSA nasal carriage both in the community (8%) and in the hospital environment (19%) should alert our health professionals to the urgent need to embark on a strict preventive regime.

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Figure 1: Flowchart showing summary of findings (n=93)

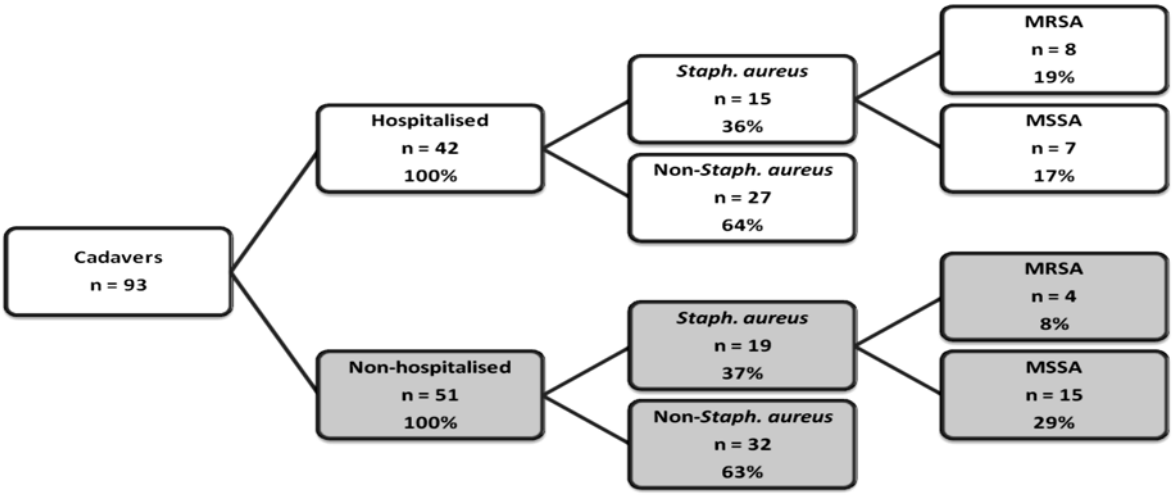

Figure 2: Incidence of resistance to other antibiotics from S. aureus isolates (n=34)