ORIGINAL ARTICLE

Frequency and Antimicrobial Susceptibility of Staphylococcus Aureus Isolated from Pus Samples of Paediatric Patients

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ABSTRACT

The formation of pus in infected wounds, ears and brain abscesses is a common problem for many patients which causes great distress in terms of discomfort, delayed healing and significantly increased healthcare cost. The aim of the study was to find out the frequency and antimicrobial susceptibility of *Staphylococcus aureus* isolated from pus samples. The study was conducted at the Microbiology Department of The Children's Hospital and Institute of Child Health, Lahore during January 2009 to January 2010. A total number of 618 pus samples collected from infected wounds, ears and brain abscesses, were analyzed during the study period. *Staphylococcus aureus* was identified on the basis of colony morphology, Gram's stain, catalase and DNase test. Out of 420 positive cultures 177 (42%) were *Staphylococcus aureus* which showed antimicrobial susceptibility to vancomycin (100%) followed by co-amoxiclav (84%), teicoplanin (82%), fusidic Acid (78.5%), cefotaxime (73%), amoxicillin (71%), erythromycin (67.7%), methicillin (66.7%), amikacin (65%), ampicillin (32%).

Key words: *Stapylococcus aureus*, Antimicrobial susceptibility, Pus samples

INTRODUCTION

Pus is a whitish yellow or yellow substance made primarily of dead white blood cells and dead bacteria; normally found in region of bacterial infections. It is produced during inflammatory pyogenic bacterial infections[1]. Accumulation of pus in an enclosed tissue space is known as abscess. A visible collection of pus within or breath the epidermis, on the other hand, is known as a collection of pustules or pimple[2]. Pus is produced from the dead and living cells which travel into the intercellular spaces around the affected cells[3]. Our skin normally protects from infections of pus producing bacteria. But sometimes these stubborn invaders penetrate the skin's defenses. They can sneak in through a wound or come in the form of skin disease, such as cyst or acne[4]. There are different microbial species which are responsible for the pus formation in wounds, ear infections and brain abscess. These include different bacterial and fungal species. The most commonly found pus producing bacteria are S*taphylococcus aureus*[5] *Staphylococcus aureus* is Gram positive cocci which is a facultative anaerobe. It is commonly present on skin flora and in nasal passage. It is one of the most common causes of nosocomial infections and can also cause post-operative infections[6].

Methicillin Resistant *Staphylococcus aureus* (MRSA) are resistant to a large group of antibiotics which contain beta-lactam ring such as penicillin and standard penicillin-related antibiotics.[6] Excessive use of penicillin antibiotics over the years has led to the development of resistant strains of bacteria that are no longer killed by penicillin type antibiotics. MRSA infections are difficult to treat in humans and these strains are often referred as super bugs[7]. *Staphylococcal* resistance to penicillin is mediated by penicillinase which cleaves the beta lactam ring rendering the antibiotic ineffective. Vancomycin and teicoplanin are glycopeptides antibiotics used to treat MRSA infections. The objective of the study was to find out the frequency and antimicrobial susceptibility of *Staphylococcus aureus* isolated from various pus samples.

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METHODOLOGY

This cross sectional observational study was conducted in the Microbiology Department of The Children's Hospital and Institute of Child Health Lahore, Pakistan, from January 2009 to January 2010. The pus swabs received during the study period were streaked on Blood and MacConkey agar plates and incubated at 37^{o}C for overnight. *Staphylococcus aureus* were identified on the basis of colony morphology, Gram stain, Catalase and DNase test[9].

The isolated *Staphylococcus aureus* species were processed for antimicrobial susceptibility testing to various antibiotics *in vitro* using the Kirby-Bauer disc diffusion method. A suspension of each bacterial strain was made according to the 0.5 McFarland turbidity standards and an even lawn of bacteria was made on Muller Hinton agar. The antibiotic discs of

amikacin (30µg), ampicillin (5µg), co-amoxiclav (20/10µg), cefotaxime (30µg), erythromycin (30µg), fusidic acid (5µg), teicoplanin (30µg), methicillin (30µg), vancomycin (30µg), and amoxicillin (30µg) were placed on the Mueller-Hinton agar plates and incubated at 37°C overnight. After overnight incubation the diameter of each zone of inhibition was measured in mm. The antimicrobial susceptibility testing results were noted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines[10].

RESULTS

A total number of 618 pus samples collected from infected wounds, infected ears and brain abscesses, were analyzed during the study period of thirteen months. Out of these, 420(61%) were positive cultures while 261 (38%) samples were negative for bacterial growth. Different bacterial species were isolated from these 420 positive samples. The frequency of Gram positive bacteria among these positive samples was 213(51%) while the frequency of Gram negative bacteria was 207 (49%). The most frequently isolated organism was *Staphylococcus aureus* 177(42%), *Pseudomonas species* 61(15%), *Klebsiella species* 58(13%), *E.coli* 47(11%), *Streptococcus species* 36(9%). The rest of the bacteria were *Proteus* species 14(3.3%), *Acinetobacter* species 8(1.9%), *Enterobacter* species 14(3.3%), *Aeromonas* species 2(0.4%), *Burkholderia* species 2(0.4%) and *Ralstonia* species 1(0.2%).

Staphylococcus aureus (n=177) showed highest susceptibility to vancomycin (100.00%) followed by co-amoxiclav (84%), teicoplanin (82%), fusidic Acid (78.5%), cefotaxime (73%), amoxicillin (71%), erythromycin (67.7%), methicillin (66.7%), amikacin (65%) and ampicillin (32%). There were 33.3% MRSA strains.

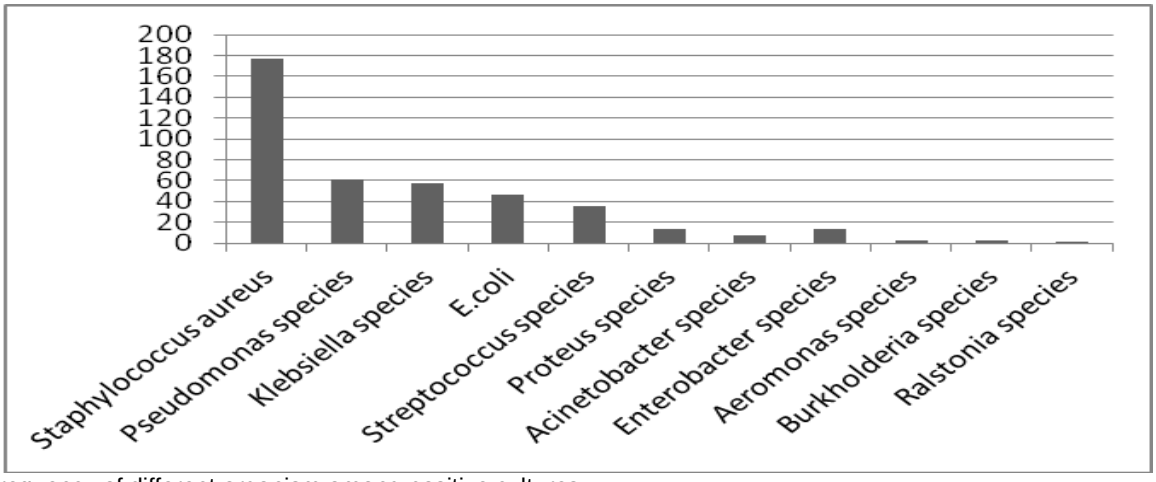

Fig 1: Frequency of different organism among positive cultures

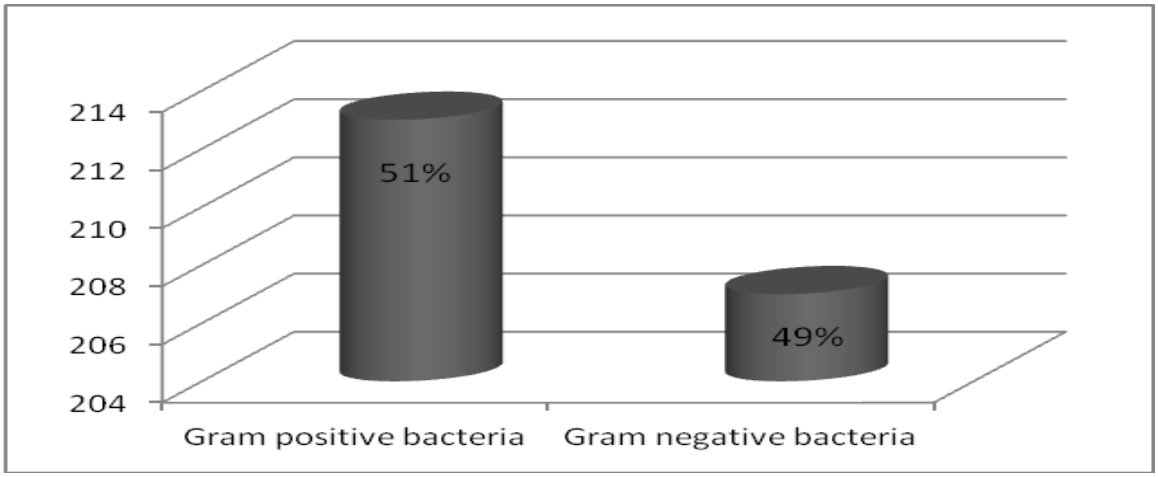

Fig 2: Frequency of Gram positive and Gram negative bacteria among positive samples

Table 1: Antimicrobial susceptibility of *Staphylococcus aureus* (n=177)

Antibiotics	Sensitive	Resistance
	No. (%)	No. (%)
Amikacin (AK)	116 (65)	61 (34.5)
Ampicillin (AMP)	57 (32)	120 (68)
Co-amoxiclav (AMC)	149 (84)	28 (16)
Cefotaxime (CTX)	130 (73)	47 (26.5)
Erythromycin (E)	120 (67.7)	57 (32.2)
Fusidic Acid (FD)	139 (78.5)	38 (21.4)
Teicoplanin (TEC)	145 (82)	32 (18)
Methicillin (MET)	118 (66.7)	59 (33.3)
Vancomycin (VA)	177 (100)	0 (0)
Amoxicillin (AML)	127 (71)	50 (28)

DISCUSSION

This study provides the data about different bacterial species isolated from pus samples and their susceptibility to various antibiotics of current use. The frequency of Gram positive organisms was found to be 51% whereas the frequency of Gram negative organisms was 49%. In a study conducted at PIMS, Islamabad the occurrence of Gram positive organisms in pus samples was 46% and 42% in 1998 and 1999 respectively, which is similar to the results of present study. Whereas the prevalence of Gram negative organisms was found to be 35% in the same period.

Among different bacterial species *Staphylococcus aureus* is recognized as an important bacterial pathogen contributing towards hospital infections, globally[11]. The frequency of *Staphylococcus aureus* in the present study was found to be 42%, followed by *Pseudomonas* spp. 15%, *Klebsiella* spp. 13%, *Escherichia coli* 11%, and *Streptococcus* spp. 8%. The rest of the bacteria, *Proteus* spp. 3.33%, *Acinetobacter* spp. 1.9%, *Enterobacter* spp. 3.33%, *Aeromonas* spp. 0.4%, *Burkholderia* spp. 0.4% and *Ralstonia* spp. 0.2% were in small number. High prevalence rate of *Staphylococcus aureus* has been reported from pus samples at National Institute of Health Islamabad, Pakistan[12]. There were 75 pus samples yielded 44% incidence of *Staphylococcus aureus* is one of the leading causes of wound infections, ears and brain abscesses and its incidence has been increased during recent decades in hospitals[11] and these results are similar with the results of our study. The next most prevalent organisms in the present study that are *Pseudomonas* species, *Klebsiella* species and *E. coli* are in line with the other studies[13,14]

In the current study the most effective antibiotic against *Staphylococcus aureus* was found to be vancomycin (100%). Some other studies showed a similar result for *Saphylococcus aureus* with 100% sensitivity against vancomycin[15]. Co-amoxiclav, teicoplanin and fusidic acid also showed good antimicrobial susceptibility pattern of 84%, 82%, and 78.5% respectively for *Staphylococcus aureus*. In other studies co-amoxiclav showed 98.9% and fusidic acid 88.5% antimicrobial susceptibility which supports the result of our study[16,17].

In current studies cefotaxime showed 73% antimicrobial susceptibility to *Staphylococcus aureus*. A research work done in 2002 reported 97.8% sensitivity against cefotaxime for the same organism[18]. Few antibiotics showed satisfactory results for *Staphylococcus aureus* which are amoxicillin (71%), erythromycin (67.7%), methicillin (66.7%), amikacin (65%) and ampicillin (32%). In different studies isolates of *Staphylococcus aureus* were found to be 63% sensitive to ampicillin 89.23% to amikacin.[19]These results are similar to current research work. The frequency of MRSA in our study was 33.3% which is close to a study carried out at Mayo Hospital where it was 38.5%[20] A study conducted in the Military Hospital Rawalpindi reported slightly higher results of 42.01% for MRSA[21]

Pus can be formed in wounds, ears and brain abscesses by the action of organisms described in this study. Although pus formation cannot be completely eliminated, a reduction in its infection rate to a minimum level could have significant benefits, by reducing wastage of health care resources and by controlled and accurate use of antibiotics[22]. A continued monitoring of susceptibility pattern needs to be carried out in individual settings to detect the true burden of antibiotic resistance in organisms and to prevent their further emergence by controlled and judicious use of antibiotics. In the present study vancomycin and teicoplanin remained effective antibiotics for the treatment of MRSA.

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