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Changing trends of pyogenic microorganisms in a tertiary care hospital

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ABSTRACT

Objective: To determine the predominant microorganisms responsible for pyogenic infections and prospective monitoring of the changing trends in their antibiotic susceptibility profile.

Methodology: A cross sectional study was conducted at the Department of Microbiology, Rawal Institute of Health Sciences, Islamabad, from April 2015 to February 2016. Pus and pus swab samples were randomly collected from different body sites both from the males and females from any age group. Patients who were already taking antibiotics, those on steroids or suffering from chronic illnesses were excluded from the study. Inoculation of the samples on the respective culture media was done followed by Gram stain, biochemical reactions and antibiotic susceptibility testing carried out by Kirby Bauer disc diffusion method and its interpretation as per Clinical Laboratory and Standards Institute (CLSI) guidelines. Data was collected on preformed table and analysis was done by using SPSS version 21.

Results: 127 pus samples were analyzed, out of which 103 yielded a positive culture. Staphylococcus aureus/Methicillin resistant Staphylococcus aureus (MRSA) was the commonest organism among the Gram positive organisms which showed maximum susceptibility to vancomycin (100%) and Linezolid (92%). Among the Gram negative organisms Escherichia coli was the commonest followed by Pseudomonas aeruginosa and other organisms.

Conclusion: The changing trends of pyogenic organisms have to be monitored prospectively to counter the global epidemic and to detect the constantly increasing burden of antibiotic resistance leading to it.

Keywords: Pus, Staphylococcus aureus, MRSA.

Introduction

Pus formation is the characteristic finding of both local and systemic inflammation due to pyogenic organisms.[1] Human skin and soft tissue infections (SSTIs) usually occur after trauma, burn wounds and surgical procedures[2] resulting in the production of pus, which could be due to both aerobic and anaerobic bacteria more commonly in hospital settings. These pyogenic infections lead to significant morbidity. Pus, a whitish yellow liquid, is formed as a result of body's defense mechanism produced during an inflammatory pyogenic infection.[3] Antibiotic resistance is a serious problem being faced globally and has the potential to drag the world into pre-antibiotic era, the basic reason behind this global threat of emerging antibiotic resistance is the wide spread and inadvertent

use of antibiotics.[4] Development of resistance to antimicrobial drugs is a particular aspect of general evolutionary change in bacteria, which is unavoidable.[5] Over the last decade the coagulase positive Staphylococcus i.e. Staphylococcus aureus has been found to be the predominant organism in pyogenic infections,[5] which may either be endogenous or exogenous. Prevalence of Methicillin resistant Staphylococcus aureus (MRSA) regarding hospital discharges has doubled and since 2004 the incidence of MRSA regarding skin and soft tissue infections has tripled.[6] Not only the infections caused by MRSA are associated with longer hospital stays, but also are an important cause of increased financial burden on the economy of the society.[6] Methicillin resistant Staphylococcus aureus is one of the commonest globally faced problems not restricted to any geographical distribution[7], being faced both in the health care facilities, sports facilities, clinics, as well as in the community.[7] Two types of MRSA strains are known, those responsible for causing infections in the hospital settings, known as the hospital-acquired MRSA (HA-MRSA) are responsible for the infections which result from implanted devices and are acquired from the healthcare settings.[6] The other type of strain being the community-acquired MRSA (CA-MRSA), because of their association with the community and they also present in people who serve as asymptomatic carriers.[7] Gram positive organisms like Methicillin resistant Staphylococcus aureus (MRSA) and the multidrug resistant Gram negative bacterial strains such as Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa are the major organisms associated with pyogenic infections especially in the hospital settings.[8,9] Over the past many years multiple studies have been conducted to assess the bacterial profile and the antibiotic susceptibility pattern in pus samples in order to start off with an empirical treatment of patients till the culture and sensitivity reports are available.[10]

Methodology

It was a cross sectional study conducted at the Rawal Institute of Health Sciences, Islamabad, Pakistan for eleven months from April 2015 to February 2016. Pus and pus swab samples were randomly collected from different body sites, both from the males and females, from any age group and whether from the outpatient department or admitted patients were included in the study. However, patients who had taken any antibiotic whether local or systemic or patients who were taking steroids or were suffering from any chronic illnesses like diabetes mellitus, chronic renal failure or any other major illness were excluded from the study. Pus or pus swabs were collected aseptically for microbiological isolation of the pathogenic bacterium/bacteria responsible. Data collected was carefully recorded in a preformed table and was analyzed through Microsoft Excel.

Received pus samples were inoculated on Blood agar and MacConkey agar plates followed by incubation at 35°C for 18-24 hours, both aerobically and anaerobically before being reported as negative. After incubation for the required time, culture plates were examined for the presence of any bacterial growth. Gram stain was performed to categorize the bacteria. Gram positive cocci were further evaluated by Catalase and Coagulase tests. For Gram negative bacteria, Oxidase test was done, followed by identification by biochemical tests (API20E, API20NE). Antimicrobial susceptibility testing of Staphylococcus aureus was carried out according to Kirby Bauer Disc Diffusion method and results were interpreted according to Clinical Laboratory and Standards Institute (CLSI) guidelines.[11] A suspension of 0.5 McFarland turbidity standards was made followed by forming an even lawn of the bacterium on Muller Hinton agar. The antibiotic discs of amikacin (30μg), ampicillin (5μg), co-amoxiclav (20/10μg), cefotaxime (30μg), erythromycin (30μg), cefoxitin (30μg), vancomycin (30μg), linezolid (30μg) and fusidic acid (5μg) were placed on Mueller Hinton agar plates and incubated at 35°C overnight. After overnight incubation the diameter of each zone of inhibition was measured in 'mm' for resistance and sensitivity according to CLSI guidelines. Statistical analysis of the data was done by using the Statistical Package for Social Sciences version 21 (SPSS 21). Frequencies (n) and percentages (%) were calculated for qualitative data.

Results

A total of 127 pus and pus swab samples were collected from April 2015 to February 2016. Out of these 103 showed positive cultures. Frequency n (%) of different organisms in positive cultures is shown in Figure 1. Frequency n (%) of Gram positive organisms was found to be higher i.e. 62 (60.19%) whereas that of Gram negative organism was 39 (37.86%). The remaining 2 (1.94%) were Candida species as shown in Figure 1. Out of a total of 62 (60.19%) Gram positive organisms, *S. aureus* was the organism isolated in highest number i.e.

51 (82.25%), out of which 26 isolates (41.93) were reported to be MRSA as in Figure 2.
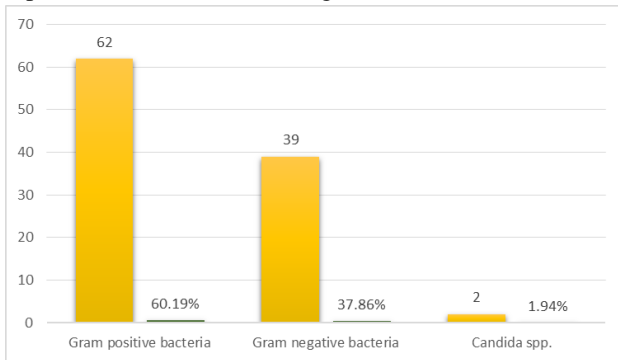

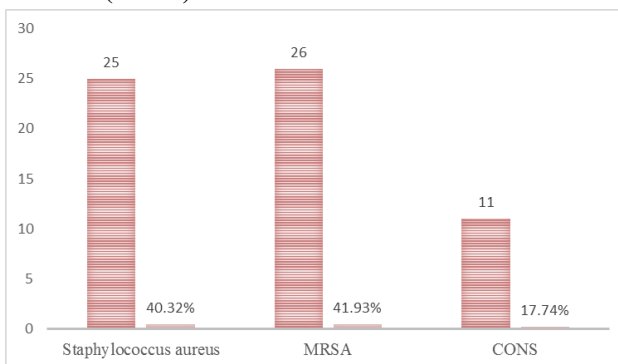**Figure 1. Frequency of organisms among positive cultures (n=103)**

Figure 2. Frequency of Gram positive organisms n=62 (60.19%)

Out of a total of 39 (37.86%) Gram negative organisms Escherichia coli and Pseudomonas aeruginosa yielded the highest number being 12 (30.76%) and 9 (23.07%) respectively followed by the rest. (Figure 3).
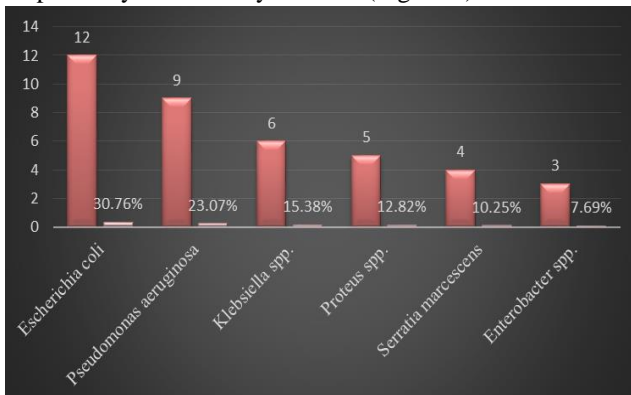

Figure 3. Frequency of Gram negative organisms n=39(37.86%)

Gender wise distribution of the isolates in our study yielded maximum being isolated from the males 68 (66.01%) as compared to females 35 (33.98%), whereas the age wise distribution of the isolates yielded maximum being isolated from 21 to 30 years, the mean for which

was calculated to be 12.88 and a standard deviation of ± 20.38. Antibiotic susceptibility of *Staphylococcus aureus* isolates is shown in the table which showed highest susceptibility to Vancomycin (100%) and linezolid (92%).

Table I: Antimicrobial susceptibility of *Staphylococcus aureus* (n= 51)

Antibiotic discs applied	Sensitive n (%)
Linezolid	47(92%)
Ampicillin	28(55%)
Erythromycin	37(66%)
Cefoxitin	25(49%)
Vancomycin	51(100%)
Co-amoxiclav	40(79%)
Cefotaxime	39(76%)
Fusidic acid	39(77%)
Amikacin	33(65%)

Discussion

Bacterial pathogens isolation rate was 81.1% in this study. Among the total positive cultures (62) 60.19% were Gram positive whereas (39) 37.86% were Gram negative which is contrary to Zubair *et al.,* 2010 [12], who reported a dominance of Gram negative bacteria in their study. On the other hand our results are very much in accordance with Mulu *et al.,*[13] who also reported the Gram positive organisms to be the predominant group and Staphylococcus aureus being the predominant organism as reported in our study.

Frequency of MRSA was calculated in this study as 41.93% which is almost the same as reported in a study by Arif *et al.,*[14] at the Military Hospital Rawalpindi, who reported it to be 42.01%, but much higher than reported in a study from PAF Hospital, Sargodha where 22.3% clinical isolates of Staphylococci were MRSA.[15] Concordant with our results, Staphylococcus aureus and MRSA were reported to be one of the major causes of soft tissue infections by Dryden *et al*.[16]

Our results of Gram negative organisms Escherichia coli and Pseudomonas aeruginosa were different from Basu *et al.,*[17] who reported Pseudomonas as the predominant Gram negative bacterial isolate.

Khanam *et al*.,[5] also came up with exactly similar results as in our study reporting the Gram positive organisms to be the predominant group and Staphylococcus aureus being the predominant organism in that group.[5] They also reported Escherichia coli followed by Pseudomonas aeruginosa being the predominant Gram negative organisms after Staphylococcus aureus, responsible for the production of pus followed by other organisms[5] as reported in our study. These results were highly

concordant with our study both in terms of the predominant group of the organisms as well the predominant organisms isolated from these groups.[5] Trojan et al, reported Escherichia coli to be the predominant organism responsible for the production of pus, in contrast to our study in which Staphylococcus aureus was reported to be the most frequent organism. According to Trojan *et al*., Staphylococcus aureus was reported as the second most predominant organism responsible for the production of pus followed again by other Gram negative organisms.[18]

Distribution of the isolates in different age groups yielded maximum isolates from 21 to 30 years of age, in our study, which matches with Dagnachew Muluye *et al*.,[19] maximum isolates of the positive cultures were from the males rather than females which is similar to the results in the study conducted by Duggal *et al*.,[20] Regarding the distribution of the isolates reported by Khanam *et al*., the predominant age group was also the same being 21 to 30 years as reported in our study and 21 to 40 years of age as reported in their study, although with a slightly wider range. [5]

Regarding the antibiotic susceptibility of Staphylococcus aureus the predominant organism out of the predominating group, it showed 100% susceptibility to Vancomycin which is highly concordant with many other studies including Trojan *et al*. [18] and Dagnachew Muluye *et al*.,[19] contrary to both of which, the sensitivity pattern reported by both of them showed all their isolates to be 100% susceptible to linezolid as well, however in our study 92 % of the Staphylococcus aureus isolates were found to be susceptible to linezolid. Khanam *et al*., also reported Staphylococcus aureus isolated in their study to be 100% susceptible to vancomycin [5] as reported in our study.

Conclusion

The changing trends of pyogenic organisms have to be monitored prospectively to counter the global epidemic and to detect the constantly increasing burden of antibiotic resistance leading to it.

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