ORIGINAL ARTICLE

Prevalence and antibiotic susceptibility of methicillin resistant Staphylococcus aureus and Acinetobacter baumannii in clinical samples from intensive care unit patients in a tertiary care hospital at Peshawar (Pakistan)

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ABSTRACT

Background & Objectives: Multi-drug resistance in Staphylococcus aureus and Acinetobacter baumannii (A baumannii) can cause a wide range of infections, including bacteremia, pneumonia, urinary tract infection, peritonitis etc., which can lead to substantial morbidity and mortality, particularly in the ICU settings. These organisms have been shown to be increasingly resistant to a large group of antibiotics, especially β-lactam antibiotics. The aim of the present study was to determine the prevalence and antibiotic susceptibility of methicillin resistant Staphylococcus aureus (MRSA) and A baumannii in patients admitted in Intensive Care Units (ICUs) of a tertiary care hospital in Peshawar, Pakistan.

Methodology: A total of 518 bacterial isolates were collected from different ICUs during the period from 1st November 2012 to 30 November 2013. Media, reagents and kits used for bacterial culture and analysis included blood agar, MacConkey's agar, mannitol salt agar, DNAase and Api Biomerieux 10s (France). Antibiotic cefoxitin was used to check whether strains of Staphylococcus aureus were methicillin resistant or sensitive. The antibiotic susceptibility testing was performed by Mueller Hinton agar (MHA) by disc diffusion method.

Results: Out of a total of 518 cultures obtained, 42(8.1%) were MRSA positive and 33(6.37%) were positive for A baumannii. Vancomycin [40(95.23%)] and minocycline [33(78.57%)] were the most effective drugs against MRSA, while colistin [33 (100%)] and minocycline [20 (60.6%)] were the most effective against A baumannii.

Conclusions: There is an increased frequency of multi-drug resistant Staphylococcus aureus and Acinetobacter baumannii (A baumannii) among patients in the ICU setting which calls for continuous surveillance to determine prevalence and effective antibiotic susceptibility of these bacteria.

Key words: Multi-drug resistance; Methicillin resistant staphylococcus aureus; MRSA; Acinetobacter baumannii; Antibiotic susceptibility

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INTRODUCTION

Hospital-acquired infections are a major cause of morbidity and mortality in ICU patients.[1] These are associated with considerable cost expenditure, increased use of prescription antibiotics and prolonged hospital stay, which all may contribute to further ICU-acquired infections.[2-4] The major causative factors are frequent exposure of ICU patients to invasive therapeutic procedures like endotracheal intubation with mechanical ventilation, nasotracheal intubation, intravenous lines, central venous lines and urinary catheterization leading to device-associated nosocomial infections.[5] This, along with limited availability of ICU beds in developing countries

like Pakistan, compounds the problems associated with nosocomial infections.

The ICU is an area of considerable antibiotic use in which antibiotic-resistant organisms are prevalent.[6] The distribution of reported causative organisms of nosocomial infections may vary across countries and even between units, according to patient case mix, sites of infection, antibiotic protocols, infection control practices, and local ecology and resistance patterns.[7] Global data have suggested a high prevalence of colonization by both MRSA and A baumannii in the ICUs.[8]

For proper management of ICU-infections, it is important to have updated knowledge about prevalence of the causative agents and their antimicrobial sensitivity/ susceptibility patterns in institution-specific ICUs.[9] Additionally, the high prevalence of resistance among these organisms and lack of data from developing regions like Peshawar supports the need for increased surveillance of patients in the long term intensive care environment in Peshawar region. This study was thus carried out to determine the prevalent microorganisms in ICU patients in a tertiary care hospital in Peshawar and also determine the antimicrobial susceptibility profile of the isolates to the commonly used antibiotics.

METHODOLOGY

After approval from the hospital ethical committee, a total of 518 bacterial isolates were collected from ICUs of RMI during a one year period from 1st November 2012 to 1st November 2013. The sample size was calculated to enable the detection of a statistically significant risk ratio of 1.5 or more with a power of 90%. Intensive care facilities included in the study were a 14 bedded Coronary Care Unit (CCU), an 8 bedded Surgical Intensive Care Unit (SICU) and a 12 bedded Medical Intensive Care Unit (MICU). Total number of samples received from all ICUs was 518, which included 35 from CCU, 269 from SICU and 214 from MICU (Table 4). These included samples taken from sputum, urine, catheter tips, pleural, pericardial or ascitic fluid, pus, blood, cerebrospinal fluid, tissue and bronchial or tracheal secretions which were subsequently cultured. Medias used for bacterial culture were blood agar, MacConkeys agar, mannitol salt agar, DNAase and Api Biomerieux 10s (France). Antibiotic cefoxitin was used to check whether strains were methicillin resistant or sensitive in cases of staphylococcus aureus. The antibiotic susceptibility testing was performed by Mueller Hinton Agar (MHA) by disc diffusion method.

RESULTS

A total of 518 bacterial isolates were collected from different ICU patients. 42 samples out of a total of 518 cultures obtained were MRSA positive (8.1%) while 33 (6.37%) tested positive for A baumannii (Table1). The antibiotic susceptibility profile showed that vancomycin and minocycline were the most effective drugs against MRSA while colistin and minocycline were the most effective against A baumannii.

Prevalence of multi-drug resistant A baumannii and MRSA in different ICUs and specimens:

Among the 518 samples, the highest prevalence of MRSA was observed in the CCU (8.57%) with the highest titer recorded from wound pus swabs (66.6%). There were no cases of A baumannii recorded from CCU. Highest frequency of A baumannii positive isolates was recorded in SICU (10.4%) with the majority of positive cultures obtained from bronchial and tracheal secretions (44.89%). MRSA was the prevalent organism obtained from MICU (8.41%) with majority of positive isolates obtained from blood cultures (52.17%) (Table 2-6).

Antibiotic susceptibility profile of MRSA and A baumannii:

MRSA exhibited the highest sensitivity to vancomycin [40 (95.23%)] followed by minocycline [33 (78.57%)], but was relatively resistant to the cephalosporins and poorly sensitive to the β-lactam penicillin drugs. A baumannii showed 100% sensitivity to colistin [33 (100%)] and also showed good sensitivity to minocycline [20 (60.6%)] and doxycycline [11 (33.3%)]. Again high resistance was shown against cephalosporins and penicillin group of drugs. Overall, vancomycin was found to be most effective against MRSA and colistin was most effective against A baumannii.

DISCUSSION

Hospital-acquired infections are most commonly associated with invasive medical devices or surgical procedures. In the present study MRSA was found to be the most prevalent organism recovered from the total samples received from the different ICUs (n=42, 8.1%)

Table 1: Positive bacterial isolates according to ICU type

	CCU	SICU	MICU	TOTAL
Total cultures received	35	269	214	518
MRSA positive n(%)	3(8.57)	21(7.80)	18 (8.41)	42 (8.1)
A baumannii positive n(%)	0	28 (10.4)	5 (2.33)	33 (6.37)

Table 2: Specimen types received from each ICU

Specimen types	CCU	SICU	MICU	Total
Sputum	3	12	8	23
Urine	9	17	50	76
Catheter tip	3	12	1	16
Fluid (pleural/pericardial/ascitic)	8	18	7	33
Pus/wound/swab	3	62	7	72
Blood	8	78	111	197
Bronchial/tracheal secretions	1	47	20	68
CSF	0	8	10	18
Tissue	0	15	0	15
Total specimens received	35	269	214	N=518

n=number of each type of specimen received

N=Total number of samples received from all ICUs

Table 3: Bacterial isolates in different specimen types in CCU

Specimens received	Total specimens	Positive		
		MRSA	A baumannii	Total
Sputum	3	1	0	1
Urine	9	0	0	0
Catheter tip	3	0	0	0
Fluid(pleural/pericardial/ascitic)	8	0	0	2
Pus/wound/swab	3	2	0	0
Blood	8	0	0	0
Bronchial/tracheal secretions	1	0	0	0
CSF	0	0	0	0
Tissue	0	0	0	0
Total	N=35	n=3(8.57)	n=0	n=3(8.57)

N=Total number of cultures received from CCU

n(%)=number (percentage) of positive bacterial isolates

Table 4: Bacterial isolates in different specimen types in SICU

Specimens received	Total specimens	Positive		
		MRSA	A baumannii	Total
Sputum	12	1	2	3
Urine	17	0	0	0
Catheter tip	12	4	1	5
Fluid(pleural/pericardial/ascitic)	18	0	0	0
Pus/wound/swab	62	5	8	13
Blood	78	1	5	6
Bronchial/tracheal secretions	47	10	12	22
CSF	8	0	0	0
Tissue	15	0	0	0
Total	N=269	n=21(7.8)	n=28(10.4)	n=49(18.2)

N=Total number of cultures received from SICU

n(%)=number (percentage) of positive bacterial isolates

Table 5: Bacterial isolates in different specimen types in MICU

Specimens received	Total specimens	Positive		
		MRSA	A baumannii	Total
Sputum	8	1	0	0
Urine	50	0	0	0
Catheter tip	1	0	0	0
Fluid(pleural/pericardial/ascitic)	7	2	0	0
Pus/wound/swab	7	1	1	0
Blood	111	10	2	0
Bronchial/tracheal secretions	20	4	2	0
CSF	10	0	0	0
Tissue	0	0	0	0
Total	N=214	n=18(8.41)	n=5(2.33)	n=23(10.7)

N=Total number of cultures received from MICU

n(%)=number (percentage) of positive bacterial isolates

with the highest prevalence rate found in CCU (8.57%). Acinetobacter baumannii was found to be the most frequent cause of nosocomial infections in the SICU (10.4%). Multi drug resistant bacteria have emerged as particularly resistant organisms in intensive care units (ICUs), and this is probably related, at least in part, to the increasingly invasive diagnostic and therapeutic procedures used in hospital ICUs in recent years. Prevalence patterns varies from study to study and in different geographical locations. In our study, prevalence rates compared to data from other hospitals in Pakistan was considerably lower; probably reflecting better institutionalized infection control practices, early surveillance and appropriate antibiotic selection. The highest prevalence of infections due to MRSA was observed in pus wound swabs received from CCU whereas highest A baumannii count was recovered from specimens of bronchial/tracheal secretions received from SICU. MRSA was the prevalent organism obtained from MICU (8.41%) with majority of positive isolates obtained from blood cultures (52.17%). Antibiotic susceptibility profile of MRSA and A baumannii showed that MRSA exhibited the highest sensitivity to vancomycin (40, 95.23%) followed by minocycline (33, 78.57%) but was relatively resistant to the cephalosporins and poorly sensitive to the β-lactam penicillin drugs. A baumannii showed 100% sensitivity to colistin (33, 100%) and also showed sensitivity to minocycline (20, 60.6%) and doxycycline (11, 33.3%). Again high resistance was shown against cephalosporin and penicillin group of drugs.

Multidrug-resistant A baumannii and MRSA are emerging as common hospital-and community-acquired infection that are difficult to treat. Being very resistant and highly aggressive these organism infect patients with weakened defenses particularly ICU patients and those with invasive devices.[10]Bacterial nosocomial pneumonia episodes occur more frequently in patients with medical admission, MRSA and A baumannii bacterial infections and prolonged mechanical ventilation, and are independently associated with higher mortality rates.[11]Recent data from the U.S. National Healthcare Safety Network indicate that gram negative bacteria are responsible for more than 30% of hospital-acquired infections, and these bacteria predominate in cases of ventilator-associated pneumonia (47%) and urinary tract infections (45%).[12]In intensive care units (ICUs) in the United States, gram-negative bacteria account for about 70% of these types of infections, and similar data are reported from other parts of the world.[13]

Infections caused by gram-negative bacteria have features that are of particular concern. These organisms are highly efficient at up-regulating or acquiring genes that code for mechanisms of antibiotic drug resistance, especially in the presence of antibiotic selection pressure. Furthermore, a plethora of resistance mechanisms exists, often using multiple mechanisms against the same antibiotic or a single mechanism to affect multiple antibiotics. Compounding the problem of antimicrobial-drug resistance is the immediate threat of a reduction in the discovery and development of new antibiotics. Several factors have contributed to this decline, including the increasing challenges of screening for new compounds, the high capital costs and long time required for drug development, the growing complexity of designing and performing definitive clinical trials, and the concern about reduced drug longevity due to the emergence of resistance. As a consequence, a perfect storm has been created with regard to these infections: increasing drug resistance in the absence of new drug development.[14]In large surveillance studies conducted by Siegel RE, between 5 and 10% of cases of ICU-acquired pneumonia were found to be due to A baumannii. A baumannii is an occasional cause of UTI, being responsible for 1.6% of ICU-acquired UTIs. A baumannii has been reported to be a more common cause of ICU-acquired bloodstream

infection than of non-ICU-ward infection (1.6% versus 0.9% of bloodstream infections, respectively, in those locations). Crude mortality overall from A baumannii bloodstream infection was 34.0% to 43.4% in the ICU and 16.3% outside the ICU.[15]

In a large multicenter study done in Pakistan by Hafiz S. et. al where multiple clinical isolates were collected from 8 laboratories all over Pakistan i.e. Karachi, Peshawar, Lahore, Sukkur, Islamabad, Quetta, Mirpur and Azad Kashmir, 42% of the isolates were found to be Methicillin resistant staphylococcus aureus (MRSA) while no vancomycin resistance was encountered which compares significantly with our study which revealed a prevalence of MRSA as 8.1% of which approximately 5% that were resistant to vancomycin. MRSA in the study by Hafiz et al was seen in the local population with frequencies varying between 2- 61% among the major cities of the country.[16]In another study in Pakistan, A baumannii prevalence was 79.3%. 68.2% isolates were MDR and out of them 44.1% were sensitive only to polymyxin and 18.6% tocotrimoxazole.[17] This compares significantly with results obtained in our study where the prevalence of A baumannii was considerably lower (6.3%) which may well be due to better sanitary infection control practices.

In recent literature from Pakistan, the frequency of A baumannii in various clinical samples taken from a tertiary care hospital in Lahore was 52%, once again much higher than the results obtained in our study (6.3%), with maximum number isolated from pus of infected wounds in orthopedic, surgical wards and intensive care unit. Here the results were comparable as the highest titer of A baumannii in our study was also obtained from the surgical intensive care unit (10.4%). These isolates were resistant to commonly used antibiotics**.** [18]**.** Patients admitted in intensive care unit are at more risk of acquiring nosocomial infection from different sources. In Pakistan, one study conducted in the intensive care unit setting by Shaikh JM et. al, the frequency of nosocomial infection was found to be 29.13%. Respiratory tract infection was seen in 30.1%, urinary tract infection in 39.1% and blood stream infection in 23.7% of patients. Other infections identified were skin, soft tissue, wound and gastrointestinal tract infections. It is suggested that proper nursing care, sterilization and disinfection of instruments and equipment and careful handling of invasive procedures are the best tool to control these life threatening infections.[19]

Akhtar N. in his study regarding multidrug resistant organism infection in ICUs recognized that the higher resistance in these patients is probably due to different antibiotic prescribing practices that can vary enormously from region to region and even within hospital specific units. Lower prevalence rates as found in our study possibly may be also due to better prescription practices at our hospital as only physicians at consultant level are allowed to prescribe antibiotics or any required medication to admitted as well as out patients. High resistance rate to the most commonly used antibiotics is most likely due to misuse, overuse and over-the-counter availability of these antibiotics. Actively addressing the problem of excessive use of broad-spectrum antibiotics can help prevent the establishment of endemic outbreaks of MDR resistant bacterial strains that are known to cause nosocomialinfection.[20]

Because of decreasing susceptibility rates of pathogens, especially ICU-acquired strains, to conventional antibiotics and a significant correlation with the length of ICU stay, intensivists should consider patient's length of ICU admission to initiate optimized empirical antibiotic therapy. Epidemiology and antimicrobial resistance patterns among commonly encountered bacteria associated with infections and colonization in intensive care units may vary diversely.

Tana R. et al. found that he most common ICU-acquired strains were A baumannii (19.5%), Pseudomonas aeruginosa (15.6%), Stenotrophomonas maltophilia (11.5%), Staphylococcus aureus (10.7%), Enterococcus spp. (10.6%), and Klebsiella pneumonia (9.7%). There were significant differences in the susceptibility rates of Gram-negative bacteria to different antibiotics with the length of stay, especially the susceptibility of A baumannii to imipenem [23.8% (ICU-acquired) vs. 44.4% (ICU-on-admission), $p < 0.001$] and meropenem (24.1% vs. 37.8%, $p < 0.001$). Furthermore, decreased susceptibility rates of A baumannii and P. aeruginosa to carbapenems were correlated with an extended ICU stay ($p < 0.05$)**.**[21]

In a study by Begum S. et al[22]., the prevalence of MDRs was reported 100% among A baumannii. The antibiotic susceptibility profile showed that minocycline and tigecycline were the most effective drugs against A baumannii. A baumannii exhibited the highest resistance 100% against cephalosporins, carbapenems, and β-lactam inhibitors. Among aminoglycosides, amikacin showed 100% resistance, while tobramycin was found to be effective against A baumannii with (39.6%) resistance. Among fluoroquinolones, ciprofloxacin showed more resistance as compared to sparfloxacin. This compares with our sensitivity and susceptibility results which showed that although A baumannii although it was indeed resistant to the penicillin and cephalosporin groups but that it was 100 % sensitive to colistin. Thus far, carbapenems have been thought of as the agents of choice for serious A baumannii infections. However, although these drugs are still active against the vast majority of A baumannii strains worldwide, the clinical utility of this class of antimicrobial is increasingly being jeopardized by the emergence of both enzymatic and membrane based mechanisms of resistance. However, results regarding MRSA susceptibility profile in

our study showed alarmingly that although vancomycin was highly sensitive (95.2%) yet it was not 100 % sensitive denoting that approximately 5% of the MRSA were vancomycin resistant or intermediate (VRSA/VISA). The emergence of a vancomycin resistant strain of staphylococcus aureus could well compound the already existing burden of multidrug resistant bacteria that is on a local as well as global rise. More studies are required to provide for earlier detection and surveillance as well as treatment strategies for this new emerging strain of MDR bacteria.

CONCLUSION

Realizing the considerably lower prevalence of MDR bacteria at this tertiary hospital in Peshawar (MRSA 8.1%, A Baumannii 6.3%) lends itself to the primary goals for the control of multi drug resistant MRSA and Acinetobacter infection. These are recognition of its presence in hospitals or long-term care facilities at an early stage, controlling spread aggressively, and preventing the establishment of endemic strains. By instituting simple but effective control measures like hand washing before and after patient contact and actively addressing the issue of excessive use of broad-spectrum antibiotics can help prevent the establishment of multi drug resistant bacterial strains that are known to cause nosocomial infection.

CONFLICTS OF INTEREST: None declared.

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