Original Research Article

Multidrug Resistant Gram Positive Pathogens with Special Reference to MRSA and Biofilm Production in ICU Patients: Recurrent Challenge for Clinicians

Richa Gupta[1*], Abida Malik[1], Meher Rizvi[1], Moied Ahmed[2] and Afreen Hashmi[1]

[1]Department of Microbiology, Anaesthesiology, JNMC, AMU, Aligarh, India
[2]Department of Anaesthesiology, JNMC, AMU, Aligarh, India

*Corresponding author

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A B S T R A C T

Gram positive pathogens mainly *Staphylococcus aureus*, *Enterococcus* and *Coagulase Negative Staphylococcus* are hospital acquired (HA) pathogens. HA strains are usually multidrug resistant (MDR). Monitoring of these strains is essential in order to control their spread in the hospital environment and transmission to the community. The objectives were to assess the prevalence of Gram positive pathogens in ICU patients and their drug resistance profile. Prevalence of methicillin Resistant *Staphylococcus aureus* (MRSA) was assessed. Health care workers (HCW) were screened for MRSA. Samples were collected aseptically from 150 ICU patients from February 2012 to November 2013. Culture, identification and antimicrobial susceptibility was performed according to CLSI guidelines. Pernasal swabs of HCW were collected for MRSA screening. Biofilm production among isolates was tested by the tube method. Among 143 bacterial isolates, 22 (13.8%) were gram positive while majority 121 (75.6%) were Gram negative. *Staphylococcus aureus* (9.4%) was the most common Gram positive pathogen isolated followed by *Coagulase negative Staphylococcus* (*CoNS*) (18.1%) and *Enterococcus faecalis* (2.4%). Among Gram positive bacterial isolates, *S. aureus* showed maximum resistance to clindamycin (73.3%), ofloxacin (75%) and gentamycin (66.7%). Sixty percent resistance was observed for oxacillin, levofloxacin and erythromycin each. *Staphylococcus aureus* isolates identified as MRSA were 66.7% and 60% by Oxacillin disc and Cefoxitin disc diffusion test respectively. Prevalence of MRSA was highest among surgical site infections(27.2%) followed by blood stream infections (20.7%)All the gram positive isolates (100%) were MDR. 13.4% of the HCW were MRSA carriers.None of the isolates were found resistant to vancomycin. Among the 22 bacterial isolates, 12 (54.5 %) were biofilm producers and all of them were MRSA. Resistance of Gram positive pathogens to commonly used antibiotics is alarmingly high. HCW are an important source of transmission of infection between hospitalized patients and they should acclimatize to proper hand washing and other simple infection control practices to inhibit such transmission.

Introduction

Throughout the world multi-drug resistant nosocomial infections are one of the leading causes of death and morbidity amongst hospitalized patients, accounting a major burden on patients and public health system of any country (Ducel *et al.,* 2002).

Intensive care unit (ICU) is one of the potential sources of nosocomial infections even in countries where extensive infection control measures are routinely implemented. The international study of infections in ICU, which was conducted in 2007, demonstrated that the patients who had longer ICU stays had higher rates of infection, especially infections due to resistant *Staphylococci, Acinetobacter, Pseudomonas species, Candida species* (Radji *et al.,* 2011).

The rate of nosocomial infections in the ICU is rising, mainly because of increasing use of invasive procedures which are performed in the ICU. The therapeutic interventions which are associated with infectious complications include indwelling catheters, sophisticated life support, intravenous fluid therapy, prosthetic devices, immunosuppressive therapy, and use of broad spectrum antibiotics leading to a spectrum of multi-drug resistant pathogens, which contributed to the evolution of the problem of nosocomial infections (Tullu *et al.,* 1998)

Moreover, the ICU mortality of infectious patients is more than twice that of non-infected patients (Vincent *et al.,* 2009).

Antibiotic resistance, a global concern, is particularly pressing in developing nations, including India (Ganguly *et al.,* 2011). Antibiotic overuse and misuse partly due to incorrect diagnosis, irrational and counterfeit antibiotic market combinations, and irregular consumption due to either wrong prescription or poor compliance all contribute to the widespread drug resistance among the hospital acquired organisms. The patterns of organisms causing infections and their antibiotic resistance pattern vary widely from one country to another, as well as from one hospital to other.

Presently, India lacks any local or national level surveillance program, to guide the stakeholders on actual prevalence of resistance (Wattal *et al.,* 2011)

The aim of the present study was to identify the prevalence of predominant multidrug resistant gram positive pathogens with special reference to MRSA and biofilm production in ICU patients.

Material and Methods

The present study was carried out among ICU patients, JNMC, AMU, Aligarh. Samples were collected aseptically from 150 ICU patients from February 2012 to November 2013. Depending on the clinical suspicions, laboratory samples like urine, sputum, pus, swab, blood, body fluids, Foley's catheter tips, ET tips, CVP line tips were collected from the patients.

Identification of all causative microorganisms was performed by standard microbiological methods. Susceptibility testing was performed using Kirby Bauer disk diffusion method according to CLSI guidelines (CLSI, 2007). *Staphylococcus* species that were resistant to 1-µg oxacillin disc and 30-µg cefoxitin disk were considered as MRSA positive (CLSI, 2007). Pernasal swabs of HCW were collected for MRSA screening. Biofilm production was tested by test tube method (Hassan *et al.,* 2011).

Other information regarding the patient including age, gender, date of admission was also collected from the case records of the patients.

Results and Discussion

Among 143 bacterial isolates, 22 (13.8%) were Gram positive while majority 121 (75.6%) were Gram negative. *Staphylococcus aureus* (9.4%) was the most common Gram positive pathogen isolated followed by *Coagulase negative Staphylococcus* (*CoNS*) (5.8%) and *Enterococcus faecalis* (2.4%) (Table 1).

Among Gram positive bacterial isolates, *S. aureus* showed maximum resistance to clindamycin (73.3%), ofloxacin (75%) and gentamycin(66.7%). Sixty percent resistance was observed for oxacillin, levofloxacin and erythromycin each (Fig.1).

Staphylococcus aureus isolates identified as MRSA were 66.7% and 60% by Oxacillin disc and Cefoxitin disc diffusion test respectively. Prevalence of MRSA was highest among surgical site infections (27.2%) followed by blood stream infections (20.7%) (Table 3).

All the gram positive isolates (100%) were MDR. None of the isolates were found resistant to vancomycin (Table 2).

13.4% of the HCW were MRSA carriers. Among 22 bacterial isolates, 12 (54.5%) were biofilm producers and all of them were MRSA (Fig. 2).

Healthcare Associated Infections (HAIs) are an important health problem in terms of morbidities, mortalities and economic consequences, world-wide (Meric *et al.,* 2005). They are especially important in intensive care units (ICUs) where they have a five-fold higher incidence rate compared to the general inpatient population (Ewans *et al.,* 1999). This is due to the increased use of medical instruments such as mechanical ventilators, monitoring devices, blood and urine catheters and also high resistance of the microorganisms isolated from ICUs patients to most commonly used antibiotics, which in turn is a result of overt use of broad-spectrum antibacterial agents`

The important reservoirs of MRSA in hospitals/ institutions are infected or colonized patients and transient hand carriage is the predominant mode for patient to patient transmission. In India, the significance of MRSA has been recognized relatively late and epidemic strains of these MRSA strains are usually resistant to several antibiotics. In our study the predominant organisms were *Staphylococcus aureus* (9.4%) was the most common Gram positive pathogen isolated followed by *Coagulase negative Staphylococcus* (*CoNS*) (5.8%) and *Enterococcus faecalis* (2.4%). These results are comparable with the findings of (Mohanty *et al.,* 2004).

In the present study, the antibiotic resistance pattern among Gram positive bacterial isolates, *S. aureus* showed maximum resistance to clindamycin (73.3%), ofloxacin (75%) and gentamycin (66.7%). Sixty percent resistance was observed for oxacillin, levofloxacin and erythromycin each, amikacin (26.7%), sparfloxacin and tobramycin (46.7%). All the isolates were sensitive to vancomycin. Similar observations were made by Khan *et al.* (2011) who observed resistance for Amikacin (73.5%), Ciprofloxacin 83.5, Clindamycin 87.79/78.26, Erythromycin 65(%), Gentamycin 70 (%), Levofloxacin 12.35/6.42, Ofloxacin (24.6%).However they found all the strains were sensitive to Linezolid and vancomycin.

Having the ability of biofilm-formation decrease their susceptibility to antibiotics. *Staphylococcus aureus* is known to form biofilms on different surfaces. In fact biofilms can resist antibiotic concentration 10-10,000 folds higher than those required to inhibit the growth of free floating bacteria (Jefferson *et al.,* 2005).

In the present study, among 22 bacterial isolates, 12 (54.5 %) were biofilm producers and all of them were MRSA. Our study was in accordance to Sasirekha *et al.* (2012) who reported 61.90% of MRSA isolates have the potential to make biofilm and in their study biofilm producing MRSA showed high resistance to almost all the groups of antibiotics compared to the biofilm non-producer. Similar were the observation of Khan *et al.* (2011). Antimicrobial resistance is an innate feature of bacterial biofilms that, in addition to the increasing rates of reported antimicrobial resistance amongst clinical

strains, may further complicate patient treatment. In our study MRSA was prevalent most among surgical site infection 27.2% followed by blood stream infection 20.2%,urinary tract infection around 6.2% and lower respiratory tract infection 5.9%.This was in accordance with the studies in Turkey and in a Children's Medical Center (Dogru *et al.,* 2010; Pourakbari *et al.,* 2012).

The present study on the bacteriological profiles of the nosocomial infections showed that the rate of such infections is high, even though it was within the reported range. The risk of development of nosocomial infections was directly related to the duration of ICU stay and the duration of the use of the indwelling catheters/tubes. The prolonged use of indwelling devices need careful prophylactic standards of microbiologic monitoring.

Table.1 Frequency of distribution of Gram positive isolates from ICU patients

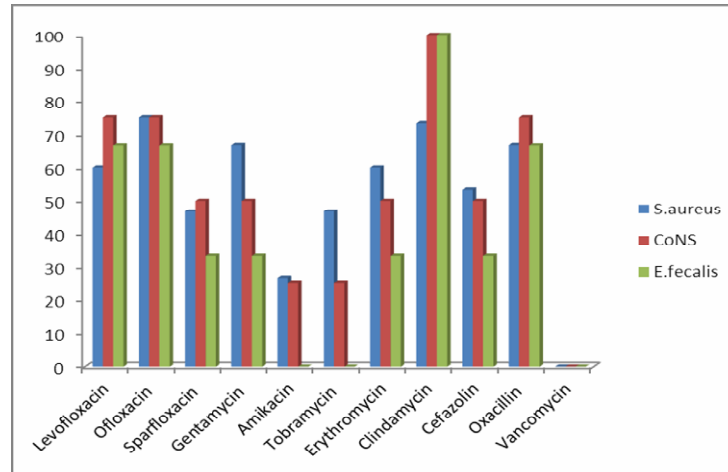| Name of the Gram Positive Isolates	Percentage of isolates
Staphylococcus aureus	22(9.4)
Coagulase negative Staphylococcus	08 (5.8)
Enterococcus faecalis	01 (2.4)

Table.2 Screening & confirmatory test results of MRSA from ICU patients

| Antimicrobial agent	*S. aureus* (%)
Oxacillin disk	10 (66.7)
Cefoxitin disk	9 (60)

Table.3 Prevalence of MRSA among different infection sites among ICU patients

Specimen	Prevalence of MRSA (%)
Surgical site infection	27.2
Blood stream infection	20.2
Urinary tract infection	6.2
Lower respiratory tract infection	5.9

Figure.1 Antimicrobial resistance pattern of Gram positive isolates from ICU patients

Figure.2 Percentage of Biofilm producing Gram positive isolates
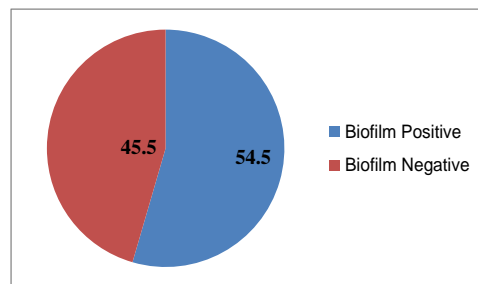
Resistance to antibiotics poses a serious and growing problem, because such resistant bacteria are becoming more difficult to treat. The empirical and the indiscriminate use of antibiotics should be avoided in order to curtail the emergence and the spread of drug resistance among nosocomial pathogens.

Reduction of nosocomial infections and antimicrobial resistance is both a challenge and goal of all ICU's around the world. Strict infection control measures like universal precautions and stringent adherence to hand washing practices, formulation of antibiotic policy, surveillance activities, might be required for the same.

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