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Bacteriological Profile of Hospital Acquired Infections with Multidrug Resistance Burden and Extended Spectrum Beta Lactamase Prevalence

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A B S T R A C T

Hospital acquired infections are one of the major causes of morbidity and mortality in hospitalized patients, leading to an enormous increase in the cost of hospital care and to the emergence of new health hazards for the community. The study aimed to determine the bacteriological profile of hospital acquired infections along with prevalence of multidrug resistance and extended spectrum beta lactamase (ESBL) enzymes amongst the isolates. A total of 180 isolates of various organisms were isolated from different clinical samples during a period of one year from January 2014 to December 2014. The antibiotic susceptibility testing of the isolates was done on Mueller Hinton agar using antibiotics from different classes which included beta lactams, aminoglycosides, macrolides and fluoroquinolones. Multidrug resistance was defined as resistance of the isolate to three or more classes of antibiotics. Extended spectrum beta lactamase detection was done in Gram negative isolates by the combined disc diffusion method. The isolates included *Staphylococcus aureus* (32.22%), *Pseudomonas aeruginosa* (20.56%), *Escherichia coli* (16.11%), Coagulase negative *Staphylococcus* (12.22%), *Klebsiella pneumoniae* (8.89%), *Acinetobacter* sp. (5.56%), *Enterococcus* sp. (2.78%) and *Proteus mirabilis* (1.67%). Out of these 180 isolates, 27 (15%) isolates were found to show multidrug resistance, *Pseudomonas aeruginosa* and *Acinetobacter* being the major multidrug resistant organisms. Out of the 95 Gram negative organisms, 39 were confirmed to be ESBL producers by phenotypic method. The study concluded that the hospital strains of microorganisms are becoming more and more resistant to the currently available antibiotics. So, the antibiotics should be used more judiciously keeping the higher antibiotics in reserve which can be a solution to multidrug resistance.

Introduction

Hospital acquired infections are one of the major causes of morbidity and mortality in hospitalized patients in the present scenario, leading directly or indirectly to an enormous increase in the cost of hospital care and to the emergence of new health hazards for the community. It has been observed that the majority of such infections emerge as a result of diagnostic and therapeutic interventions such as intravenous cannulas, indwelling catheters, sophisticated life support, intravenous fluid therapy, prosthetic devices, immunosuppressive therapy, and the use of broad spectrum antibiotics (Shalini *et al.*, 2010). The rate of hospital acquired infections varies from 2.8% to 34.6% among

hospitalized patients (Rosenthal *et al.,* 2006). A prevalence survey conducted under the auspices of WHO in 55 hospitals of 14 countries representing 4 WHO Regions (Europe, Eastern Mediterranean, South-East Asia and Western Pacific) showed an average of 8.7% of hospitalized patients had hospital acquired infections.

At any time, over 1.4 million people world-wide suffer from infectious complications acquired in hospital (Benenson *et al.,* 1995). The highest frequencies of hospital acquired infections were reported from hospitals in the Eastern Mediterranean and South-East Asia Regions (11.8% and 10.0% respectively) with a prevalence of 7.7% and 9.0% respectively in European and Western Pacific regions (Tikhomirov *et al.,* 1987). The organisms that cause hospital acquired infections are often multidrug-resistant which poses a major public health threat. The regular use of antimicrobials for treatment therapy or prophylaxis promotes the development of resistance. Extended spectrum beta lactamases are an important cause of this resistance. The detection of ESBL-producing organisms in laboratories is a critical requirement for appropriate management of patients, infection prevention and control efforts, as well for tracking these organisms in surveillance systems.

The aim of this study was to determine the bacteriological profile of hospital acquired infections along with determination of multidrug resistance (MDR) and extended spectrum beta lactamase (ESBL) enzymes amongst the isolates.

Materials and Methods

The study was conducted in the Department of Microbiology, School of Medical Sciences and Research, Sharda Hospital, over a period of 12 months, from January 2014 to December 2014.

Various clinical samples like urine, blood, pus, sputum, endotracheal secretions, and central venous line tips were collected from patients suspected to be suffering from hospital acquired infections from various wards (including orthopaedics, surgery, medicine, gynaecology, paediatrics, ENT) and ICUs and were transported immediately to the laboratory. The specimens were processed according to standard bacteriological procedures available (Mackie and McCartney, 2006). They were inoculated on Blood agar and MacConkey agar plates and the growing organisms were identified by standard techniques. Ambiguous results were confirmed by automated VITEK 2-compact system (BioMerieux, France) following the manufacturer's instructions. Antibiotic sensitivity testing was performed on Mueller Hinton agar using antibiotics from different classes - beta lactams, glycopeptides, aminoglycosides, macrolides and fluoroquinolones. Multidrug resistance (MDR) was defined as resistance of the isolate to three or more classes of antibiotics. The CLSI recommended combined disk method involving ceftazidime and cefotaxime with and without the inhibitor clavulanic acid (30 µg) was used to confirm the presence of ESBL in Gram negative isolates.

Results and Discussion

A total of 180 isolates of various organisms were isolated from different clinical samples of patients suffering from hospital acquired infections. Out of these 180 isolates, *Staphylococcus aureus* constituted the maximum proportion i.e. 58 strains (32.22%), which was followed by 37 strains of *Pseudomonas aeruginosa* (20.56%), 29 of *Escherichia coli* (16.11%), 22 of Coagulase negative Staphylococcus (12.22%), 16 of *Klebsiella pneumoniae* (8.89%), 10 strains of *Acinetobacter* sp. (5.56%) and least by those of Enterococcus species i.e. 5 strains (2.78%) and 3 of *Proteus mirabilis* (1.67%) (Fig. 1).

Out of the 180 isolates, 27 (15%) were found to show multidrug resistance which was mainly against cephalosporins, fluroquinolones and aminoglycosides. Non-fermenters ranked highest amongst the MDR organisms with 40.54% strains of *Pseudomonas aeruginosa* being MDR and 30% of *Acinetobacter*. This was followed by *E. coli*, 20.69%, *Klebsiella*, 12.50% and *S. aureus*, 1.72%. *Proteus*, Coagulase negative Staphylococcus and *Enterococcus* were not found to be multidrug resistant (Table 1).

Out of the 95 Gram negative organisms, 39 were confirmed to be ESBL producers. Amongst the ESBL producing Gram negative organisms, *E. coli* (51.72%) was found to be the predominant ESBL producer followed by *Klebsiella* (43.75%), *Pseudomonas* (37.84%), *Proteus* (33.33%) and *Acinetobacter* (20%) (Table 2).

Hospital acquired infections occur worldwide, both in the developed and developing world. They are a significant burden to patients and public health and are a major cause of death and increased morbidity in hospitalized patients. Our study therefore aimed to establish the local data on the bacteriological profile of hospital acquired infections and detection of multidrug resistance burden.

In the present study, 180 isolates were obtained from samples acquired from patients who showed signs and symptoms of infection after 48 hours of admission to the hospital.

The bacteriological profile showed that *S. aureus* (32.22%) was the predominant pathogen followed by *P. aeruginosa* (20.56%), which was the commonest non-fermenter (8.89%). The other organisms included *Acinetobacter* sp. (5.56%), *Enterococcus* sp. (2.78%) and *Proteus mirabilis* (1.67%). These results were in agreement with a study of 71 patients conducted at Post Graduate Institute of Medical Education and Research (PGIMER) in Chandigarh in which it was found that 35% of the pathogens isolated from wounds and blood of patients having nosocomial infections were *S. aureus* and 24% were *P. aeruginosa* (Sunil Saggar, 2011). Another six-month study conducted in 2001 of the intensive care units (ICUs) at All India Institute of Medical Sciences (AIIMS) in New Delhi, found that 140 of 1,253 patients (11 per cent) had 152 hospital-acquired infections, where *P. aeruginosa* made up 21% of isolates, 23% were *S. aureus*, 16% *Klebsiella* spp., 15% *Acinetobacter baumannii* and 8% *Escherichia coli* (Sunil Saggar, 2011). However, our bacteriological profile was dissimilar to the profile observed in a study in Turkey in which the most common infecting microorganism was *A. baumannii* (25.5%), followed by *P. aeruginosa* (14.2%), *K. pneumoniae* (8.6%), *E. coli* (8.5%) and *S. aureus* (8%) (Candevir *et al.*, 2011).

A study conducted in Ahmadabad also showed that the commonest organism isolated from all samples was *E. coli* (25%) followed by *Acinetobacter* sp. (15.62%), CoNS (16.40%), *Klebsiella* sp. (14.06%), *Pseudomonas* sp. (13.28%), *Candida* sp. (4.68%) which was different from our study (Zaveri *et al.*, 2012). This difference in the bacteriological profile may be due to the difference in the geographical regions and also in the type of procedures conducted and the antibiotic policies being followed in different hospitals.

In our study we observed that out of the 180 isolates responsible for nosocomial infections, 27 i.e. 15% of the isolates were multidrug resistant.

Fig.1 Bacteriological profile of hospital acquired infections - 180 isolates

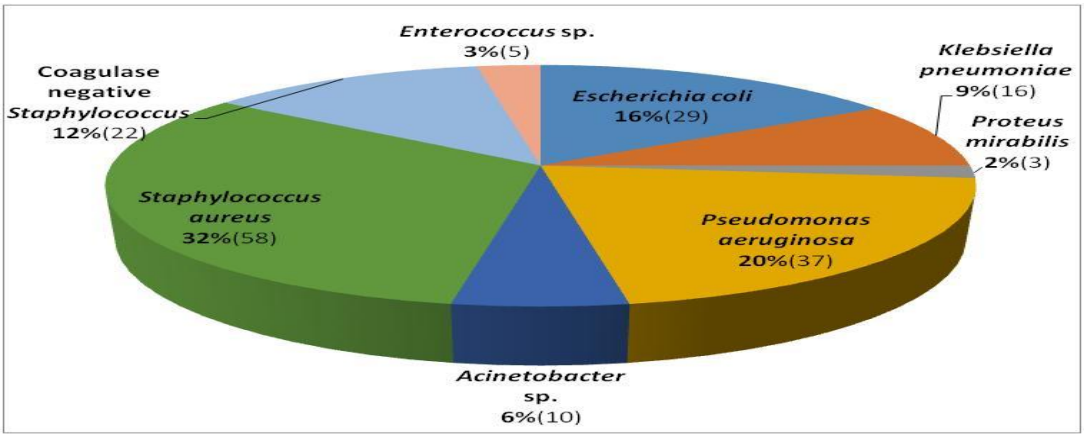

Table.1 Distribution of multidrug resistant organisms

Organism	Number (Total)	Percentage
E.coli	6 (29)	20.69
Klebsiella	2 (16)	12.50
Proteus	0 (03)	0.00
Pseudomonas	15 (37)	40.54
Acinetobacter	3 (10)	30.00
S.aureus	1 (58)	1.72

Table.2 Distribution of ESBL producing organisms

Organism	Number (Total)	Percentage
E.coli	15 (29)	51.72
Klebsiella	7 (16)	43.75
Pseudomonas	14 (37)	37.84
Acinetobacter	2 (10)	20.00
Proteus	1 (03)	33.33

Table.3 Percentage of ESBL producing organisms in various studies

ESBL producing organism	Our study, India, 2015	Bandekar *et al.*, India, 2011	Oberoi *et al.*, India, 2013	Tsering *et al.*, India, 2009	Baharullah *et al.*, Pakistan, 2013
E.coli	51.72	-	73.38	26. 15	77.77
Klebsiella	43.75	53.33	79.54	57. 14	47.36
Pseudomonas	37.84	44.74	66	32.61	46.42
Proteus	33.33	37.5	50	42.86	20
Acinetobacter	20	46. 15	60	-	

This percentage was close to that observed in a study done in Oman which showed 11.2% of the isolates to be MDR (Balkhair *et al.,* 2014). However, our results were different from those observed by Basnet who found 62.80% MDR organisms in their study which was much higher than that of ours (Basnet *et al.,* 2013).

Our study showed that it were the non-fermenters which constituted the major percentage of multidrug resistant organisms amongst all the isolates. *Pseudomonas aeruginosa* showed 40.54% MDR which was similar to that observed in a study conducted in Nepal (41.18%) (Basnet *et al.,* 2013).

However other studies done in Oman (Balkhair *et al.,* 2014) and Ahmadabad (Zaveri *et al.,* 2012) showed lower MDR in *Pseudomonas aeruginosa* i.e. 8.1% and 5.89% respectively whereas studies done in Egypt (Ahmed *et al.,* 2013) and Istanbul, Turkey (Unan *et al.,* 2000) showed a higher percentage of MDR in *Pseudomonas aeruginosa* i.e. 52% and 60% respectively.

In our study, 30% of *Acinetobacter* isolates showed multidrug resistance which was similar to the study done in Oman i.e. 32.4% (Balkhair *et al.,* 2014). However, Basnet (Basnet *et al.,* 2013) showed higher percentage of multidrug resistant *Acinetobacter* (89.19%) as against Zaveri who showed a lower percentage (10%) as compared to our study (Zaveri *et al.,* 2012).

Our study showed that 20.69% *E.coli* and 12.5% *Klesiella* were MDR. This was similar to the results of a study done in Oman which showed 18.4% and 10.3% MDR in *E.coli* and *Klebsiella* respectively (Balkhair *et al.,* 2014). Another study done in Nepal showed a higher percentage of MDR in *E.coli* and *Klebsiella* i.e. 82.60 and 36.36% respectively (Basnet *et al.,* 2013).

This difference in the multidrug resistance pattern of organisms in various studies may be due to difference in antibiotic policies being followed.

The prevalence of ESBLs in the Gram negative bacteria in our study was 41.05%. This was quite close to a study conducted in Punjab which showed 35.16% ESBL production (Oberoi *et al.,* 2013). Similar findings were reported in a study done by Bandekar which showed a high prevalence of the ESBL producers (39.8%) in burn patients (Bandekar *et al.,* 2011).

A study which was done by Harakuni reported a high prevalence of the ESBLs (74%) in ICU patients (Harakuni *et al.,* 2011). Study done by Laghawe and others reported 19.67% ESBL producers (Laghawe *et al.,* 2012). It has been proved that the prevalence of the ESBLs among the clinical isolates varies from country to country and institution to institution within the same country. In India, the prevalence rate varies in different institutions from 28 to 84% (Das *et al.,* 2001). A study from Coimbatore, Tamil Nadu, showed the presence of ESBLs to be 40% while from Nagpur this figure was 50% in urinary isolates (Babypadmini *et al.,* 2004, Tankhiwale *et al.,* 2004). Another study from New Delhi showed 68.78 % of the strains of gram negative bacteria to be ESBL producers (Mohanty *et al.,* 2005).

In our study, we found *E.coli* (51.72%) to be the maximum ESBL producer followed by *Klebsiella* (43.75%), *Pseudomonas* (37.84%), *Proteus* (33.33%) and *Acinetobacter* (20%). This was in agreement with the study done by Baharullah who also observed *E. coli* (77.77%) to be the major ESBL producer followed by *Klebsiella* (47.36%), *Pseudomonas* (46.42%) and *Proteus* (20%) (Umer *et al.,* 2013). However other studies done by Oberoi (Oberoi *et al.,* 2013),

Bandekar (Bandekar *et al.*, 2011) and Tsering (Tsering *et al.*, 2009) showed *Klebsiella* to be the major ESBL producer which was different from our study (Table 3).

There is no doubt that ESBL-producing infections are of grave concern to the medical world. They are associated with an increased morbidity and mortality and can be difficult and time consuming to identify. Coupled with the fact that prevalence rates are rising globally, including in nonhospital settings, and the dire lack of effective antimicrobial therapy, the future is tremendously concerning. Urgent work is required to develop quicker, cost-effective, and reliable diagnostic tools as well as new effective therapies. We hope that this study will form a useful reference for clinical microbiologists, physicians and others attempting to monitor the prevalence of hospital acquired infections and for the treatment of patients with such infections.

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