FREQUENCY OF MULTIDRUG RESISTANT AND EXTENSIVELY DRUG RESISTANT ORGANISMS IN TRACHEAL ASPIRATES – EXPERIENCE AT A TERTIARY CARE HOSPITAL

EJAZ A.,[1] TARAR M.R.,[2] NAEEM T.,[3] NAEEM M.A.,[4] IJAZ S.,[5] AND QURESHI M.[6]
[1-3,5,6]*Department of Pathology, Shalamar Medical and Dental College and Shalamar Teaching Hospital, University of Health Sciences,* [4]*School of Business and Economics, University of Management and Technology, Lahore – Pakistan*

ABSTRACT

Background and Objective: The multidrug resistant (MDR) and extensively drug resistant (XDR) organisms are a common problem globally, especially in Intensive Care Units (ICUs). The objective of this study was to determine the frequency and antibiotic susceptibility pattern of organisms isolated from tracheal aspirates of ICU patients and classify these into either of the two groups.

Methods: This was a retrospective analysis of laboratory records of tracheal aspirates obtained from patients admitted in the ICU of Shalamar Teaching Hospital over a period from 01 August 2012 to 31 July 2015 submitted for culture/sensitivity. The tracheal aspirates with positive culture/sensitivity were analyzed with SPSS ver. 21 using One – way ANOVA test for each antibiotic against different bacterial species.

Results: Out of 98 samples, 79 had positive growth and 19 yielded no growth whereas 5/79 yielded two types of organisms. Overall, 84 isolates were available for analysis, 61/84 were Gram negative rods whereas 8/84 were Gram positive cocci. Fifteen had fungal growth. Based on sensitivity profile, the isolates were classified as XDR, MDR and those with normal sensitivity pattern.

Conclusions: Majority of the isolates belonged to XDR (56.5%) or MDR (37.6%) whereas only a few (5.8%) had normal sensitivity pattern. This situation is alarming for patient management and infection control/prevention in hospitals.

Key Words: Acinetobacter baumannii, Pseudomonas aeruginosa, Intensive care units, Drug resistance.

INTRODUCTION

The pathogenic bacteria are developing resistance to various antimicrobial agents and this has become a major public health issue. Over the recent years, multidrug resistant (MDR) and extensively drug resistant (XDR) organisms have increasingly been implicated in healthcare associated infections (HAIs).[1] MDR is the acquired resistance to at least one agent in three or more antimicrobial categories whereas XDR is resistance of bacterial isolates to one or more agents in all except two or less than two categories.[2]

One of the common sites of infection in hospitalized patients is endotracheal tube (ETT) particularly in ICUs where invasive interventions are common.[3] A higher rate of respiratory colonization by hospital strains in intubated patients in the ICU has been observed, thus increasing the risk of pneumonia by 6 to 20 times.[4] The presence of a device like ETT and infection by an MDR or XDR organism can contribute to major morbidity and mortality in patients.[3,5,6] The objective of our study was to determine the frequency of antibiotic susceptibility pattern of organisms isolated from tracheal aspirates of ICU patients and classify these into either of the two groups (MDR and XDR).

MATERIALS AND METHODS

This was a descriptive retrospective cross sectional analysis of lab records of tracheal aspirate samples of patients admitted in the ICU of Shalamar Teaching Hospital Lahore Pakistan. All tracheal aspirate specimens received from the ICU of Shalamar Teaching Hospital, from 01 August 2012 to 31 July 2015, were included in the study. Repeat specimens from same patient were not included in the study. The presence of an isolate and its antibiotic susceptibility pattern in 98 tracheal aspirate samples was analyzed.

The data was analyzed with SPSS ver. 21 using One – way ANOVA test for each antibiotic against different bacterial species. P-value ≤ 0.05 was considered significant.

The Samples received in sterile universal containers were cultured on Blood and Mac Conkey Agar and incubated overnight at 37°C aerobically. The isolated organisms were Gram stained and further processing

Corresponding Author: Dr. Asma Ejaz. Department of Pathology, Shalamar Medical and Dental College and Shalamar Teaching Hospital, Lahore, University of Health Sciences Lahore. Ph: + 92 (0) 3216833788 (Cell). Email: ejaz.asma11@gmail.com

was done accordingly. Based on Catalase test reactions, the Gram positive cocci were divided into either Staphylococci or Streptococci/Enterococci. The Staphylococci were further categorized as *Staphylococcus aureus* and *Staphylococcus epidermidis* based on Coagulase and DNAase tests and sensitivity to Novobiocin (5 µg) disc. The Streptococci were further processed based on their hemolytic reactions on blood agar. Grouping was done on Beta hemolytic Streptococci using Prolex™ Streptococcal Grouping Latex Kit (Prolab diagnostics Merseyside UK) according to manufacturer instructions. The Group D Streptococci were further tested on Bile Esculin Agar (Oxoid CM0888). The Gram negative bacilli were identified using Analytical Profile Index (API 20E Biomerieux Marcy-l'Etoile / France).[7] Antimicrobial susceptibility testing was performed using Kirby Bauer Technique according to CLSI 2012 guidelines.[8] A panel of 13 drugs was applied for Gram negative organisms and a panel of 12 drugs for Gram positive organisms (Table 1).

Table 1: *Panel of antibiotics used for Gram -ve and Gram +ve organisms.*

Gram Negative Panel	*Gram Positive Panel*
Ampicillin	Ampicillin
Amoxicillin – Clavulanic Acid	Cefoxitin (marker for Flucloxacillin)
Cefuroxime	Co-trimoxazole
Cefotaxime	Cefuroxime
Ceftriaxone	Erythromycin
Cefoxitin (marker for ESBL production)	Clindamycin
Co-trimoxazole	Ciprofloxacin
Ciprofloxacin	Gentamicin
Gentamicin	Fusidic Acid
Amikacin	Vancomycin
Imipenem	Teicoplanin
Meropenem	Linezolid
Colistin	

RESULTS

There were total 98 tracheal aspirate samples. Nineteen samples showed no growth and 79 showed positive growths. Five of them yielded two types of organisms. Altogether, 84 isolates were recovered from these samples. Sixty one isolates were Gram negative rods and 8 isolates were Gram positive cocci whereas 15 samples showed fungal growth (Table 2).

Acinetobacter baumannii was the commonest isolate (30.9%) and majority of them were XDR (Table 3). *Pseudomonas aeruginosa* was the second commonest with a frequency of 17.9%. All other isolates are mentioned in Table 3 in order of their frequencies.

Among bacterial isolates, XDR and MDR organisms were 56.5% and 37.6% respectively and non-MDR organisms were 5.8% (Fig. 2).

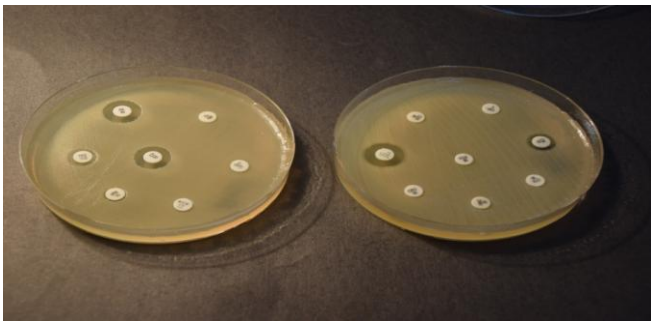

Fig. 1: *Antibiotic susceptibility pattern of one of the XDR Acinetobacter baumannii isolate.*

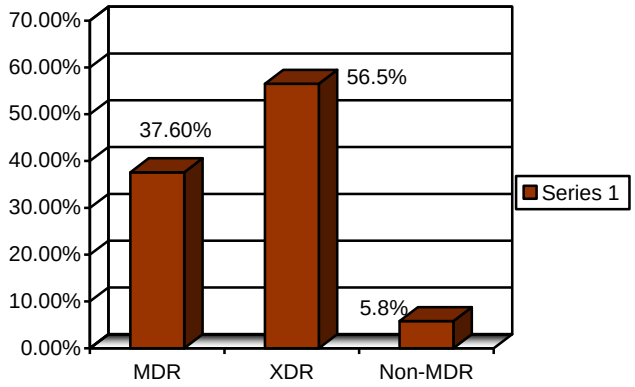

Fig. 2: *Frequency of MDR, XDR and Non-MDR/XDR isolates among pathogens from ICU patients.*

Statistical analysis for Gram Negatives: The usefulness of six most effective drugs was calculated against Gram Negative isolates. One – way ANOVA test was

Table 2: *Isolate frequency in Tracheal Aspirate samples.*

Total Samples	*Total Organisms*	*Two Types of Growth*	*Bacteria*		*Fungi*	*No Growth*
			GNR	*GPC*		
98	84	5	61 (72.6%)	8 (9.5%)	15 (17.8%)	19 (19.4%)

GNR: Gram –ve rods GPC: Gram +ve Cocci

Table 3: *Distribution of microbial isolates from Tracheal Aspirates of ICU patients.*

Type of Organism	*Frequency (%)*
Acinetobacter baumannii	26 (30.9%)
Pseudomonas aeruginosa	15 (17.9%)
Escherichia coli	10 (11.9%)
Klebsiella pneumoniae	5 (5.9%)
Staphylococcus epidermidis	6 (7.1%)
Citrobacter freundii	2 (2.4%)
Proteus mirabilis	1 (1.2%)
Serratia marcescens	1 (1.2%)
Enterobacter cloacae	1 (1.2%)
Enterococcus faecalis	1 (1.2%)
Staphylococcus aureus	1 (1.2%)
Candida albicans	15 (17.9%)

applied using the drug's sensitivity/resistance ratio within the group and among the groups. This showed that the differences among the groups were significant ($p \leq 0.05$). Subsequently, Tukey's *post hoc* comparison was run and the plot clearly showed that Colistin was the most effective drug against the Gram Negative isolates (Fig. 3).

Statistical analysis for Gram Positives: The usefulness of all drugs was calculated against Gram Positive isolates. One way ANOVA test was applied using

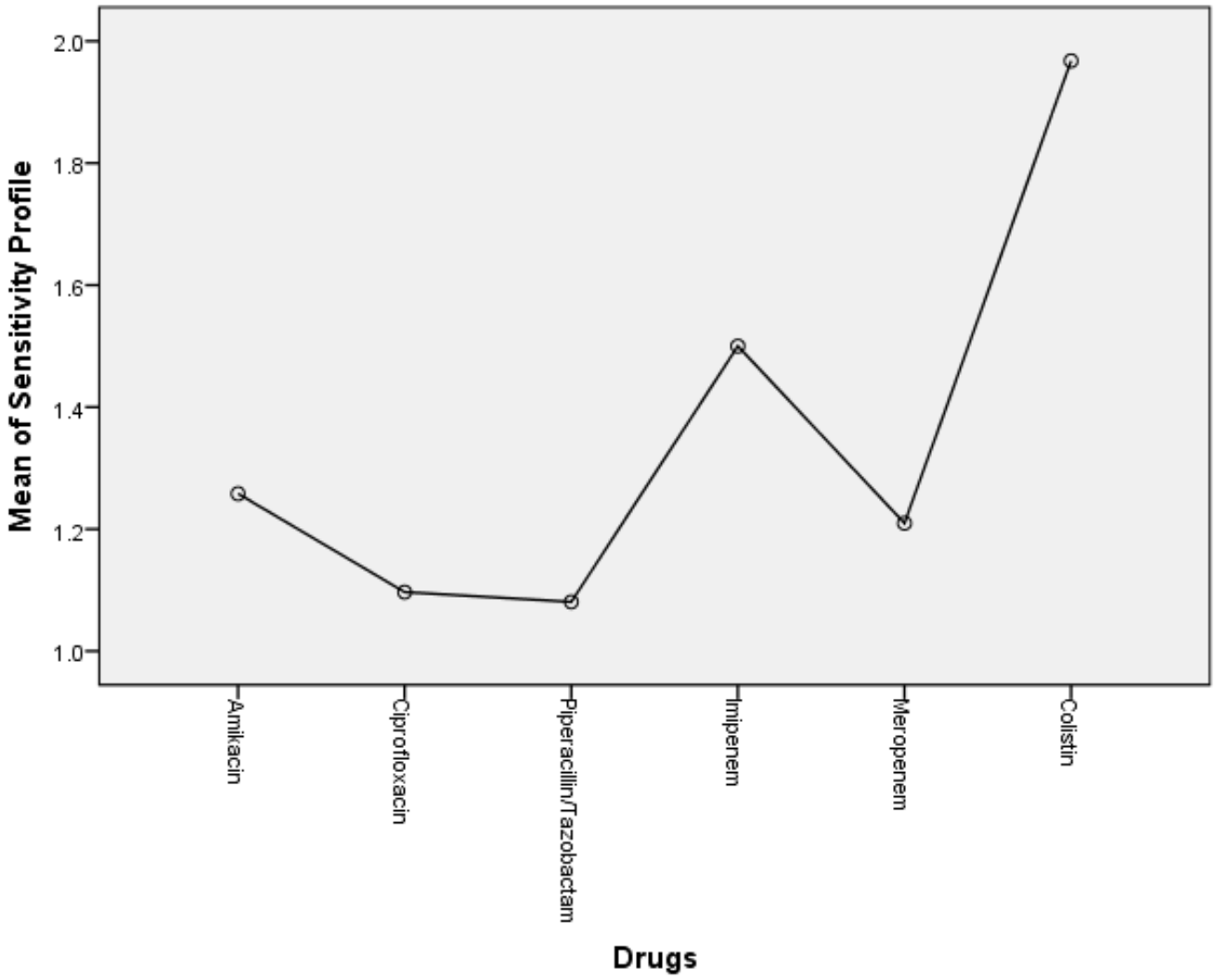

Fig. 3: *Tukey's post hoc comparison showing Colistin as the most effective drug among the Gram Negative isolates.*

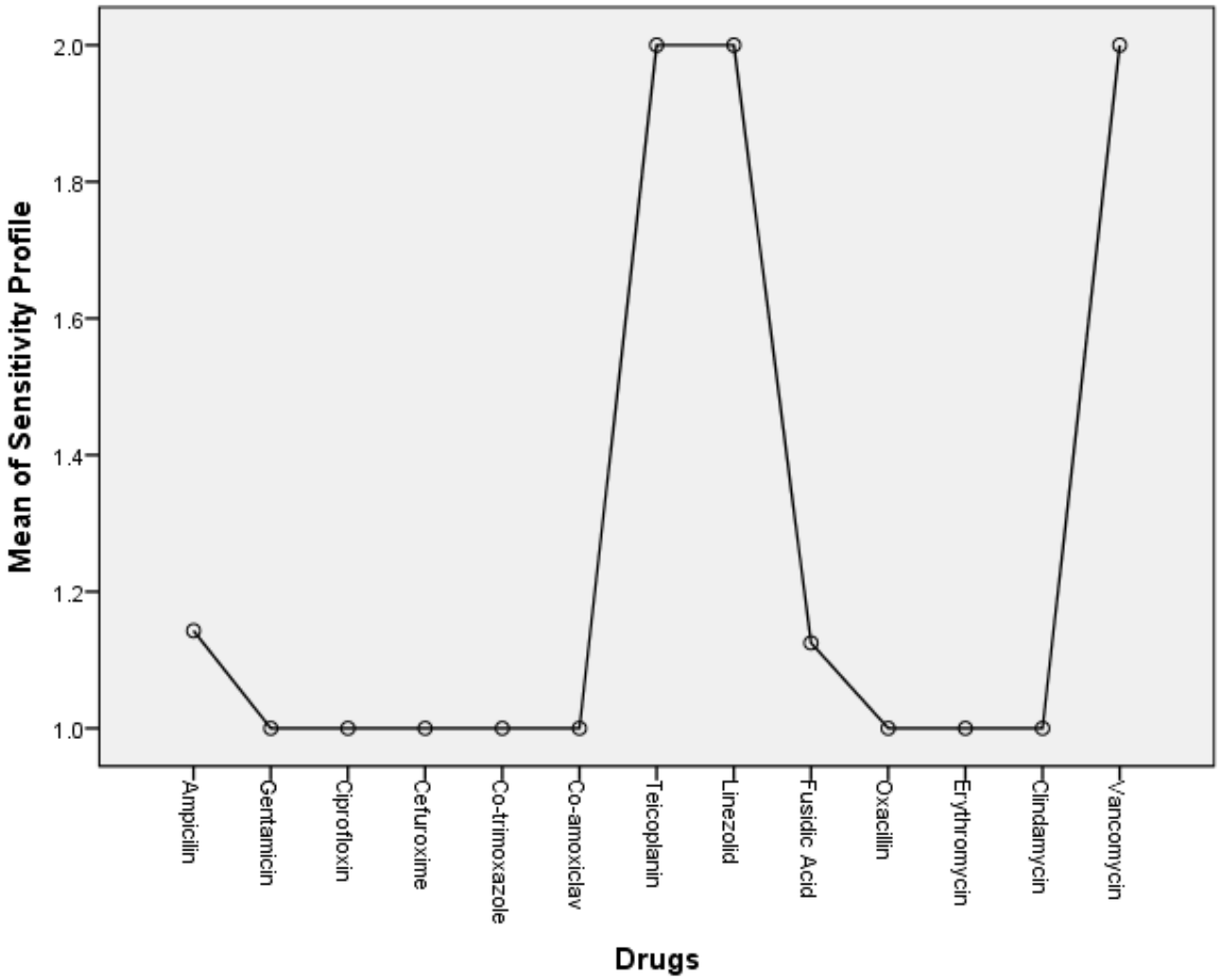

Fig. 4: *Tukey's post hoc comparison showing Vancomycin, Teicoplanin and Linezolid as the most effective drugs among the gram positive isolates.*

the drugs sensitivity/resistance ratio within the groups and among the groups. This showed that the differences among the groups were significant ($p \leq 0.05$). Subsequently, Tukey's *post hoc* comparison was run and the plot clearly showed that Vancomycin, Teicoplanin and Linezolid were the most effective drugs among the Gram positive isolates (Fig. 3). Ampicillin was useful against Enterococcus only. The Oxacillin resistance was because of the reason that most of the isolates were Coagulase negative *Staphylococci* which are usually resistant to penicillinase resistant group of antibiotics as compared to *Staphylococcus aureus*.

DISCUSSION

Antimicrobial resistance is becoming a critical issue in hospital settings, both for the management and prevention of serious infections in patients.[9] Not only is the cost of patient care rising, but considerable morbidity and mortality in patient populations is also a direct consequence.[4,6] Both Gram negative and Gram positive bacteria are contributing to this drug resistance.[6,10] With this background, we were keen to determine the frequency of multi- and extensive drug resistance in both types of bacteria isolated from tracheal aspirates of patients in the ICU of our hospital. The samples from the respiratory tract like sputum and tracheal aspirate can be contaminated by oral flora. However, tracheal aspirate is collected under aseptic conditions, making interpretation and clinical correlation easier.[11] This sample is obtained from debilitated, critically ill, or immunocompromised patient population. We decided to focus on tracheal aspirates as this was the most common sample that had been submitted from ICU patients in our set-up.

A recent study on tracheal aspirates reported 88% of their isolates to be Gram negative, *Pseudomonas aeruginosa* being the most frequent.[12] In our study, more than two third of the isolates were Gram negative

organisms; *Acinetobacter baumannii* and *Pseudomonas aeruginosa* being the commonest. Another study conducted in Iran on prevalence of bacteria in endotracheal tubes of patients showed the most prevalent organism to be *Enterobacter* species.[4] *Acinetobacter baumannii* is an important nosocomial pathogen in hospitalized patients. It is a persistent colonizer due to its ability of forming biofilm which is an important virulence factor for device associated infections.[13,14] It has been found to be more resistant to antibiotics and causes outbreaks difficult to treat HAIs in ICUs.[15] In our study, 56% of the isolates were classified as XDR and 37% were MDR. In the study referred to above, a similar trend was observed as 55% and 27% of their tracheal aspirate isolates were XDRs and MDRs respectively.[12] Interestingly, over the span of study period, we noticed a gradual increase in antibiotic resistance pattern of *Acinetobacter baumannii* with more XDR isolates being reported in 2015 compared with 2012 that were resistant to all antibiotics with the exception of Colistin (unpublished results). In a South American study, Rosales *et al* have shown that *Acinetobacter baumannii* became resistant to all antibiotics except Colistin.[13] This is an issue of great concern and a molecular study is warranted to determine the cause for this change in antibiotic sensitivity pattern. A similar trend has been reported by two groups of researchers from North America and South Korea about increasing resistance of *Acinetobacter baumannii* in hospital environment leading to serious difficulties in treating HAIs.[16,17] In our study, *Pseudomonas aeruginosa* did not show the change in resistance pattern as observed for *Acinetobacter baumannii.*

The Gram positive organisms in our patients constituted only nine percent, which was similar to that reported elsewhere.[12] In our study, Vancomycin, Teicoplanin and Linezolid were the most effective drugs for Gram positive isolates similar to a recent study reporting the three drugs as the most affective among enterococcal isolates.[18] The cases due to Gram positive organisms especially *Staphylococcus aureus* may be predicted with a Gram stain analysis of the tracheal aspirate which has a negative predictive value of 92.8% and the specificity is even higher (97.8%).[19] The result should be communicated to the treating physician so that a decision to include Gram positive cover empirically can be made at the earliest.

It is ***concluded*** that the lower respiratory tract infections in hospitalized patients, especially by Gram negative organisms, are on the increase and the majority of the isolates belong to XDR and MDR category. This situation is important not only for the management but also for prevention of HAIs. Institutional guidelines for the empirical therapy need to be laid down to provide adequate antibiotic cover for these patients. In addition, enhanced infection prevention activities are warranted to control the spread of these organisms in the hospital in general and between the patients in particular.

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Authors' Contribution

A.E. Data collection, literature search, material and methods and results writing, contributed to discussion writing. **M.R.T.** Conceived the idea, literature search, contributed to discussion writing. **T.N.** statistical analysis, literature search, contributed to discussion writing. **M.A.N.** statistical analysis of the data and writing the results based on the analysis. **S.I.** Data collection, literature search. **M.Q.:** Head of the Microbiology Unit, final review before submission.

Conflict of Interest: None.

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