Antimicrobial susceptibility pattern of Bacteria isolated from Tracheal secretions in Intensive Care Units admitted Patients of Lahore General Hospital

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Declaration of conflicting interests

The Authors declares that there is no conflict of interest.

ABSTRACT

Background: Critically ill patients of Intensive care units (ICUs) are always at greater risk for acquiring hospital associated infections with multidrug resistant, (MDR) microorganisms. One of the commonest and important nosocomial infections, which is acquired by ICUs admitted patients, who are intubated for mechanical ventilation is ventilator-associated pneumonia (VAP). The morbidity of these patients is increasing due to invasion of MDR strains of microorganisms. The etiology of these MDR superbugs may vary according to the different ICUs settings along with patient's illness and their antibacterial treatment.

Objective: To observe Antibiogram of bacteria isolated from tracheal secretions from the patients of ICUS admitted in Lahore General Hospital (LGH).

Materials and method: This is a descriptive study carried out in Lahore General Hospital, (LGH) Lahore during Jan 2017 to Dec 2017. Total 445 samples were processed for culture and sensitivity according to standard operating procedures at Microbiology laboratory of Pathology department (PGMI, Lahore. These secretions were including the admitted patients of different ICUs of LGH. Including patients having fever $\geqslant$ 38 ˚C, WBCs count $\geqslant$ 10,000/mm^3 or $\leqslant$ 3 000/mm^3, Purulent tracheal secretions, Diffused or patchy infiltration in chest radiograph.

Results: Out of 445 samples of tracheal secretions, 365 were collected from males and 80 from females. The large numbers of samples 222/445 were collected from PINS ICUs. 370 (83%) showed positive culture growth i.e. CFU $\geqslant$ 10. 66.2% culture showed pure growth while 16.8% showed poly-microbial growth of two and three type of bacteria. The commonest bacteria were Klebsiella pneumoniae, which was isolated from tracheal secretion. The most susceptible trend was seen with combination drugs Cefeperazone- salbactam and Pipperacillin-tazobactam more than 60% of their sensitivity was observed among Gram-negative bacteria and susceptibility to Vancomycin and Linezolid was 100% among Gram-positive bacteria.

Conclusion: The present study showed trend of bacterial antimicrobial susceptibility in tracheal secretion in ICUs admitted patients of Lahore General Hospital. In order to struggle with antibiotics resistance every hospital should design a strict and applicable infection control policies, sensible use of antibiotic, education with infection control programs.

Key words: Antimicrobial susceptibility; ICUs; Klebsiella pneumonia; MDR; Tracheal secretions; VAP

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Introduction

Critically ill patients of ICUs are always at greater risk for acquiring hospital associated infections with multidrug resistant microorganisms. This is due to their prolonged hospital stay, immunocompromised profile, serious illness, use of invasive devices, catheters and prolonged use of antibiotics.[1] The frequent and unselective usage of broad-spectrum antibiotics without reporting of culture and sensitivity leads to development of these multidrug resistant superbugs in the world of microbiology and this creates problem for the treatment of ICUs patients.[2,3]

One of the commonest and important nosocomial infections, which is acquired by ICUs admitted patients, who are intubated for mechanical ventilation is ventilator-associated pneumonia (VAP). The mechanical-ventilation is one of the lifesaving practices for ICUs admitted patients but it has a greater risk of developing respiratory infections.The morbidity of these patients is increasing due to invasion of MDR strains of microorganisms. Among these superbugs, who are associated with pneumonia, are Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella spp. and Acine to bacter spp. Apart from this, ICUs patients developed multibacterial infections during their prolonged stay in hospitals. These changing floras also complicate the therapy by developing MDR and their sensitivities pattern.[4]

Family Enterobactericeae comprising of Gram-negative rods, their common prevalent bacteria have ability to develop resistance against different β-lactam agents, which attributes their resistance to broad-spectrum cephalosporin. The antimicrobials of this group were primarily administrated for treatment of ICUs patients in hospitals previously. Along with them, the bacteria also developed antimicrobial resistance to other groups of antibiotics like Trimethoprim Sulphamethoxazole, Fluoroquinolones and Aminoglycosides.[5] Similarly, MRSA strains are also one of the most important microorganisms regarding nosocomial infections in ICUs. MRSA developed due to excessive usage of antibiotics in ICUs setting. About 70% of Staphylococcus aureus isolated from ICUs are MRSA.[1]

The etiology of these MDR superbugs may vary according to the different ICUs settings along with patient's illness and their antibacterial treatment. Therefore, it is mandatory to have knowledge about bacterial pattern of the hospital ICUs settings and their local antimicrobial susceptibility pattern, which provides guidelines to the clinicians for prompt and empirical treatment with appropriate antibiotics.[6] This is achieved by the gold standard culture for identifying bacterial etiology and susceptibility pattern of them, which is the aim of the present study conducted in ICUs patients of Lahore General Hospitals on their tracheal secretions.

Materials And Methods

It was a descriptive study, the data was included from Jan to Dec 2017, total 445 numbers of endotracheal secretions were included that were sent to microbiology laboratory of PGMI/LGH for culture and sensitivity. These secretions were collected from the admitted patients of different ICUs of LGH, which includes ICUs of department of medicine, surgery, pediatrics and Punjab institute of neurosciences (PINS).

Patients of ICUs admitted for more than 48 hours of either gender and age, having fever $\geqslant$ 38 °C, whose WBCs count $\geqslant$ 10,000/mm^3 or $\leqslant$ 3 000/mm,^{3} having purulent tracheal secretions and with diffused or patchy infiltration in chest radiograph were included in our study.

Patients clinically and radiologically having signs of pneumonia before hospital admission, patients with other respiratory tract infections and immunocompromised patients were excluded from the study.

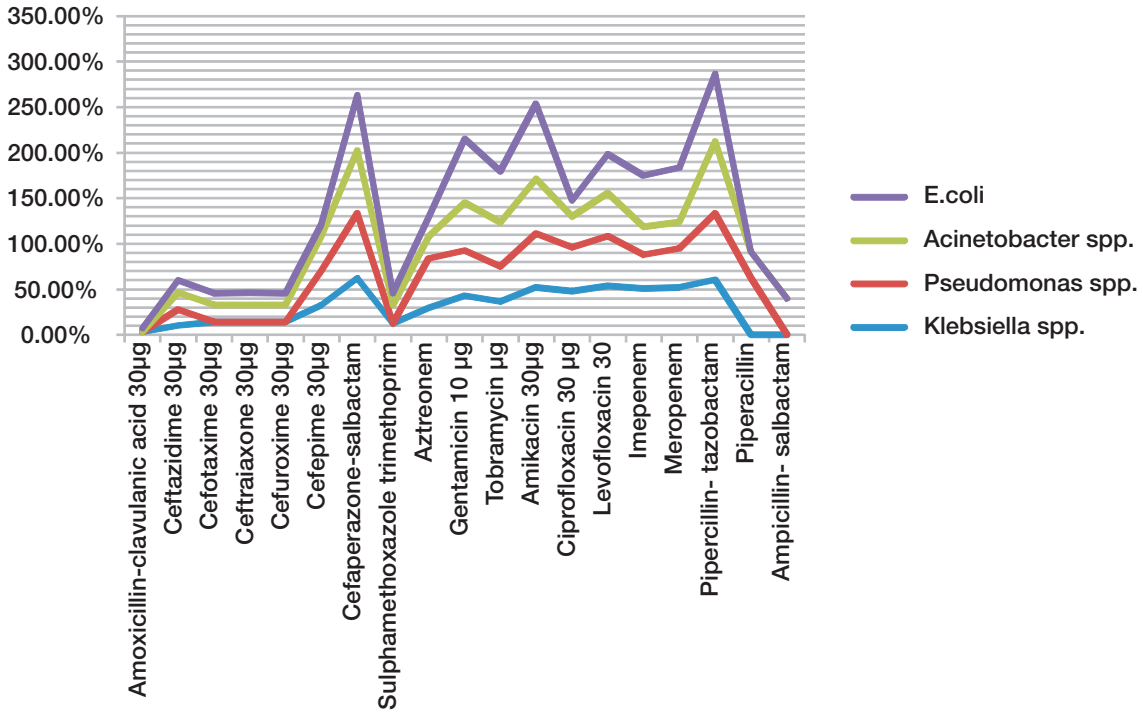
Microbiological Analysis: Gram's staining of these samples was done to rule out that whether the bacteria were a colonizer or pathogen using Q score.[70] It also provided an initial clues about the type of bacteria, whether the material was purulent or not, i.e. $\geqslant$ 25 neutrophils and $\leqslant$ 10 squamous cells per LPF. The received samples of endotracheal secretions were inoculated on blood agar and MacConkeys agar with added crystal violet and incubated for 24 hours in an incubator at 37 °C The cultures were read next day for any positive or negative growth. The culture read as semi quantitatively when growth was moderate or heavy and quantitatively when more than 10^5 colony-forming units CFU /mL of bacteria were isolated on culture. The bacteria were preliminary identified on basis of their colonial morphology, presence or absence of hemolysis on blood agar, fermenter or non-fermenter. Then Gram's staining was done to confirm whether Gram-positive or Gram-negative cocci or bacilli. Further confirmation was done by biochemical reactions like catalase, coagulase and oxidase tests etc.[8,9] The antimicrobial susceptibility testing was done on Mueller-Hinton agar by Kirby-

Bauer disc diffusion method.[10] The plates were incubated at 37°C for 24 hours and interpreted as per CLSI (Clinical and Laboratory Standards Institute 2017) guidelines.[11] The following antibiotics were used for antimicrobial susceptibility testing.

Ampicillin, amoxicillin-clavulanic acid, cefuroxime, ceftriazone, ceftazidime, cefotaxime, cefipime, sulphamethoxazole trimethoprim, Imipenem, meropenem, aztreonem, Ciprofloxacin, Levofloxacin, Amikacin, Gentamycin, tobramycin, piperacillin-tazobactam, cefaperazone-salbactam were used for Gram negative bacilli. For Pseudomonas, spp. additional pipercillin was used and ampicillin salbactam was additionally used for Accinetobacter spp. For Gram positive bacteria antibiotic disk of Linezolid, Vancomycin, Penicillin, amoxicillin clavulanic acid, Ciprofloxacin, levofloxacin, amikacin, Gentacin, tobramycin, Sulphamethoxazole trimethoprim, Erythromycin, azithromycin, clathromycin, clindamycin and cefoxitin was used for identification of MRSA (CLSI 2017). MDR are considered when organisms were not susceptible to more than one drug in at least three anti-microbial groups.[12]

Table 1: Antimicrobial susceptibility pattern of different bacterial species isolated from ICUs patients from their tracheal secretions.

Antibiotics	% Age of Sensitivity			
	Klebsiella spp N=135	Pseudomonas spp. N=110	AcinetobacterSpp. N=58	E.coli N=23
Amoxicillin-clavulanic acid30µg	2.9%	--	--	4.3%
Ceftazidime 30µg	10.2%	17.3%	18.9%	13.0%
Cefotaxime 30µg	13.9%	--	18.9%	13.0%
Ceftraiaxone 30µg	14.0%	--	19.0%	13.5%
Cefuroxime 30µg	13.9%	--	18.9%	13.0%
Cefepime 30µg	32.3%	38.0%	37.9%	13.0%
Cefaperazone-salbactam	61.7%	71.2%	68.9%	60.8%
Sulphamethoxazole trimethoprim	12.2%	--	20.6%	13.0%
Aztreonem	29.4%	53.9%	24.0%	21.0%
Gentamicin 10 µg	42.6%	49.5%	53.0%	69.5%
Tobramycin µg	36.0%	39.0%	48.0%	56.0%
Amikacin30µg	52.2%	59.0%	60.0%	82.0%
Ciprofloxacin 30 µg	47.8%	47.8%	34.0%	17.3%
Levofloxacin 30	53.9%	53.9%	47.4%	43.0%
Imepenem	50.7%	37.0%	31.0%	56.0%
Meropenem	52.2%	42.6%	29.0%	59.3%
Pipercillin-tazobactam	60.2%	73.0%	79.0%	74.0%
Piperacillin	--	62.3%	29.0%	--
Ampicillin-salbactam	--	--	39.6%	--



Graph 1: Trend of antibiotic susceptibility of Gram-negative bacteria isolated in our study.

Results

Our results showed that Gram-negative bacilli (Klebsiella pneumoniae and E.coli) were least susceptible to Amoxicillin clavulanic acid 2.9% and 4.3% respectively. They also showed high resistance to cephalosporin and Sulphamethoxazole trimethoprim including Pseudomonas aeruginosa and Acinetobacter spp. 13% to 38%. Except Pseudomonas aeruginosa, which was 53.9% susceptible to Aztreonem, Klebsiella pneumoniae, E.coli and Acinetobacter spp. showed high resistance to Aztreonem group 13 to 20.6%.

Klebsiella pneumoniae and E.coli showed good response to Carbapenems (imepenem and meropenem) above 50% of bacteria were susceptible to them. However, Pseudomonas aeruginosa and

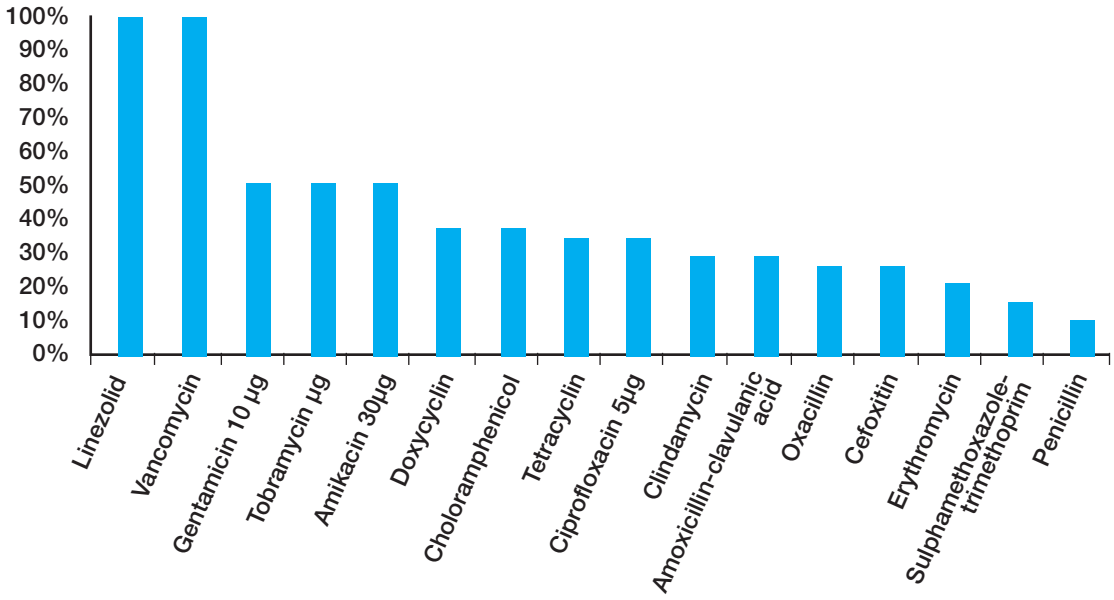


Figure 1: Antibiogram of Staphylococcus aureus showing sensitivity of different antibiotic

Acinetobacter spp. were more resistant to them less than 50% susceptibility.

All bacteria were more than 50% susceptibility to Floroquinoles and Aminoglycosides, Levofloxacin and Amikacin had better susceptibility as compared to other group members.

The most susceptible trend was seen with combination drugs Cefeperazone-salbactam and Pipperacillin-tazobactam more than 60% of their sensitivity was observed.

In our study, Staphylococcus aureus showed 100% susceptibility to Vancomycin and Linezolid. Followed by 51% susceptibility to Aminoglycosides. 27% were sensitive to cefoxitin because of which 73% of Staphylococcus aureus of our study were reported as MRSA.

Discussion

VAP is one of the major causes ofhospital-acquired pneumonia. By using 'clinical pulmonary infection score' CPIS guidelines we could reduce antimicrobial resistance in bacterial etiology of VAP.[13] In our study, all Gram-negativebacteria including Acinetobacter spp. showed high resistance to amoxicillin-clavulanic acid, cephalosporins, cotrimoxazole and Aztreonem except Pseudomonas aeruginosa, which was relatively less resistant to aztreonem (53.9% susceptible toPseudomonas spp.). These results were also consistent with the results of study conducted by Kumar et al 2011.[14] Multidrug resistance among the respiratory pathogens is an alarming concern associated with HAIs. The most common Multi drug resistant bacteria in our study wereKlebsiella pneumoniae and Acinetobacter spp. However, Pseudomonas aeruginosa is relatively more susceptible to other groups of antibiotics. These observations were also in accordance to the study conducted on MDR bacteria of LRTIs by Vishwanath et al 2013.[15] In our study Gram negative bacilli were more susceptible to cabapenems, aminoglycosides, and combination drugs of beta lactam along with beta lactamases inhibitors e.g Cefaperzone-salbactam and pipperacilli –tazobactam, more than 60% susceptibility to combination drugs was observed. Garg et al 2017 also concluded that piperacillin-tazobactem was the most susceptible antibiotic (46.7%) in VAP in their ICUs settings[13]. However, those 40% resistant strains that were resistant to these combination antimicrobials were an alarming trend and leading these MDR super bugs to extreme drug resistance (XDR) those who were resistant to all antimicrobials except Colistin and Tegicyclin.[16]

Acinetobacter spp. showed its resistance to cabapenems as compared to Gram-negative bacilli. Acinetobacter spp. is emerging as a major MDR and XDR pathogens in hospital acquired infections predominantly in VAP, also reported by Hartzell et al 2007.[17] In the present study, among carbapenems, bacteria were more resistant to imepenem as compared to meropenem. Parihar et al 2016 also documented the rising resistance of imepenem among their isolates and commented it as alarming trend.[18]

Pseudomonas aeruginosa showed 100% sensitivity to Colistin in our study. Thomas et al in 2016 also reported similar results for Pseudomonas spp. susceptibility to Colistin. Colistin is re-introduced among gram-negative bacteria for extreme drug resistant susceptibility.[19] In the present study, 73% staphylococcus aureus were MRSA, Ahmed et al 2017 also reported MRSA as a most frequent bacteria (40%) in their study conducted on VAP.[20]

Conclusion And Recommendations

In our study, high level of antibiotic resistance to more than three antimicrobial groups was observed among Gram negative as well as Gram-positive bacteria. However, combination drugs like Piperacillin-tazobactam and Cefeperazone- salbactam showed better susceptibility results yet their resistance is also observed in more than 40% of cases, which is also an alarming trend that leads to extreme drug resistance among hospital bacterial flora. Initially antimicrobial resistance was associated with hospital-acquired infections but now it is extended to community level. In order to struggle with antibiotics resistance every hospital should designed a strict and applicable infection control policies, sensible use of antibiotic, education with infection control programs to hospital personnel and promotion of hand hygiene should be practiced. Moreover, more studies should be conducted for determination of pattern of bacterial etiology and their resistance pattern should be assessed, which will be helpful for the clinicians for better management of these critical patients of ICUs.

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