ORIGINAL ARTICLE

Antimicrobial Susceptibility Profiles of Aerobic Bacteria Causing Infections at a Tertiary Care Centre

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Abstract:

Introduction: The multidrug resistant bacteria are of great concern.[1] Antimicrobial resistance poses a major threat to patient's treatment as it leads to increased morbidity and mortality, increased hospital stay, and severe economic loss to the patient and nation.[2] The pattern of bacteria causing these infections and their antimicrobial susceptibility profiles vary widely.[3] The present study was conducted to isolate aerobic bacterial pathogens from various specimen and to determine their antimicrobial sensitivity pattern. *Material and Methods:* The pathogenic aerobes from various samples were isolated. The antimicrobial sensitivity pattern was determined for these microorganisms using conventional microbiological techniques. *Results:* Skin and soft tissue infections (SSTIs) (n=1229, 60%) were the most common infections followed by lower respiratory tract infections (LRTIs) (n=471, 22.61%), urinary tract infections (UTIs) (n=255, 12.24%) and blood stream infections (BSIs) (n=130, 6.23%). The most common bacteria isolated were Klebsiella *sp*. n=641(30.77%) followed by *S. aureus* n=519, (24.91%), *Pseudomonas sp.* n=326 (15.65%), *E. coli* n=262(12.6%), *Citrobacter sp*. n=135(6.48%), *Acinatobacter sp*. n= 107(5.13%) and others n= 95(4.56%). The best drug combination for BSIs, LRTI, UTI and SSTIs was meropenem and vancomycin. The extended spectrum beta lactamases (ESBL) and methicillin resistant staphylococcus aureus (MRSA) were found approximately 40%. *Conclusion:*The results of the current study emphasize the importance of institutional antibiotic policy for the effective and timely management of patients due to increasing drug resistance profile of bacterial pathogen with regional variation.

Keywords: antimicrobial sensitivity, aerobic bacteria, multidrug resistance.

Introduction:

The multidrug resistant bacteria are of great concern especially in severely ill patients due to limited treatment options. The increasing occurrence of antimicrobial resistance (AMR) for beta-lactam group of antimicrobial is a major concern. It is mainly due to the presence of beta-lactamases. Beta-lactamases are of diverse types, of which extended spectrum beta-lactamases (ESBLs) and carbapenemases are rapidly disseminating. [1] In recent years, there is four fold rises in number of multidrug resistant organisms (MDROs) worldwide. At present, antimicrobial resistance poses a major threat to patient's treatment as it leads to increased morbidity and mortality, increased hospital stay, and severe economic loss to the patient and nation.[2] The pattern of bacteria causing these infections and their antibiogram vary widely from one region to another as well as from one hospital to other and even among the Wards and ICUs within one hospital.[3] Empiric antibiotic treatment at the beginning of the disease can even prevent the spread of the disease and may ultimately reduce morbidity and mortality due to cancer.[4] Knowledge of etiological agents of infections and their sensitivities to available drugs is of immense value to the rational selection of antimicrobial agents and to the development of antibiotic policy.[5] Several large-scale surveillance studies are being conducted to monitor AMR across the globe. Studies have reported that the burden of AMR is high in Asian countries. However, data from India regarding AMR studies is negligible. Due to this, there is a lack of information on the incidence rates and real burden of AMR in India. [6] Therefore we have decided to collect and analyze the data on antimicrobial resistance (AMR) Surveillance for our hospital. This will provide guidance on empirical treatment and formulating antibiotic policy for the patients admitted in this tertiary care centre.

Material and Methods:

This is a retrospective observational study which included a total of 2083 clinical isolates of aerobic bacteria obtained from various samples at this tertiary care centre. These were isolated from the patients who were admitted in our hospital from January 2021 to December 2021.All the data collected was unlinked anonymous. All samples submitted for isolation and AST of aerobic bacteria in bacteriology section were selected for the study. All samples submitted for anaerobic and fungal culture and AST were excluded from the study. All the isolates were processed as per standard conventional microbiological techniques. The identification and antimicrobial sensitivity testing of bacterial pathogen was done using standard methods. [7, 8, 9] We have used the standard CLSI Document M100-S28 for the interpretation of results of AST patterns of aerobic bacteria.[9] The confirmation of species of isolated pathogens was done on second day of sample processing. The pure bacterial growth was used for antimicrobial sensitivity testing. We didn't store the bacterial isolate. The AST results for both first and second line drugs were made available on third day. Antibiotic discs of HiMedia Company were used. For *S.aureus*, the antibiotics tested and reported were as follows: erythromycin (15µg), clindamycin (2µg), gentamicin (10 µg), amikacin (30 µg) ciprofloxacin (5 µg), linezolid (30 µg), nitrofurantoin (50 µg) and cotrimoxazole (1.25/23.75 µg). E strips used were vancomycin. Cefoxitin (30 µg) was used for detection of methicillin resistant staphylococcus aureus (MRSA). For gram-negative bacteria, the antibiotics were chosen

Results:

In the present study, a total of 2083 bacterial isolate were identified from lower respiratory tract infections from the following: ciprofloxacin (5 µg), norfloxacin (5 µg), amoxycillin-clavulanic acid (30 µg) (20/10 µg) , nitrofurantoin (50 µg), amikacin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftazidime clavulanic acid (30/10 µg), cefepime (30 µg), piperacillin tazobactam (100/10 mcg), tobramycin (10 µg), imipenem (10 µg), meropenem (10 µg). Extended spectrum β lactamases (ESBLs) production was detected by CLSI Phenotypic confirmatory test (disk potentiation test) using ceftazidime (30 µg), ceftazidime – clavulanic acid (30/10 µg) discs. [9] The Clinical Laboratory Standards Institute (CLSI) recommended quality control (QC) strain such as *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *K. Pneumoniae* ATCC 700603 (ESBL-positive control) were used for quality controls. The antibiotic sensitivity pattern for the bacterial pathogens was determined using standard CLSI guidelines. [9] The analysis of data was done with the use of standard SPSS analysis. As this is the retrospective observational study, ethical approval was not needed as suggested by our institutional committee. followed by LRTIs (n=471, 22.61%), UTIs (n=255, 12.24%) and BSIs (n=130, 6.23%). The most common bacteria isolated were *Klebsiella sp.* n=641(30.77%) followed by *S. aureus* n=519, (24.91%), *Pseudomonas sp.* n=326(15.65%), *E. coli* n=262(12.6%), *Citrobacter sp.* n=135(6.48%), *Acinatobacter sp.* n= 107(5.13%) and others n= 95(4.56%). (Table 1)

Table 1: Bacterial isolates identified in various infective syndromes

	E.coli	*Klebsiella sp.*	*Pseudomonas sp*	*Acinatobacter sp.*	*S. aureus*	*Citrobacter sp*	other bacteria	total
LRTIs	40	208	87	38	51	30	17	471 (22.61%)
UTIs	94	66	32	0	27	16	20	255 (12.24%)
SSIs	116	339	202	59	379	81	53	1229 (59%)
BSIs	12	28	5	10	62	8	5	130 (6.23%)
total	262 (12.6%)	641 (30.77%)	326 (15.65%)	107 (5.13%)	519 (24.91%)	135 (6.48%)	95 (4.56%)	2083

The antibiogram of gram negative bacteria showed that gentamicin, meropenem were highly sensitive and piperacillin-tazobactum, tobramycin were moderately sensitive. The lower sensitivity was observed with ceftazidime and fluroquinolones. The amoxicillin clavulanic acid was the least sensitive for gram negative isolates. The nitrofurantoin had good sensitivity for gram negative bacteria (GNB) from urine. (Table 2)

Table 2: Antimicrobial sensitivity pattern in percentage among aerobic gram negative bacteria

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Name of antimicrobial agent	Percentage sensitivity (%) of aerobic gram negative organism from different types of specimens			
	Respiratory specimen	Urine	Pus	Blood
gentamicin	82	44	53	50
meropenem	60	60	54	60
amikacin	54	44	48	50
tobramycin	50	32	57	62
piperacillin tazobactum	60	53	40	50
ceftazidime	57	NA	35	NA
ciprofloxacin	50	35	33	50
amoxicillin/ clavulanic acid	34	38	35	38
nitrofurantoin	NA	54	NA	NA

The antibiotic sensitivity pattern of GNB showed the increased number of extended spectrum beta lactamase producing organisms (ESBLs) n= 629/1564 (40.21 %). The antibiotic sensitivity test (AST) pattern of gram positive bacteria for vancomycin and linezolid was 100%. The sensitivity for gentamicin, amikacin and clindamycin was found moderate. The least sensitivity was observed for ciprofloxacin and cotrimoxazole. Gram positive organisms from urine had moderate sensitivity for nitrofurantoin. (Table 3) The antimicrobial susceptibility profile revealed the methicillin resistant *Staphylococcus aureus* (MRSA) as n= 189/519 (36.41 %).

Table 3: Antimicrobial sensitivity pattern in percentage among aerobic gram positive bacteria

Name of antimicrobial agent	Percentage sensitivity (%) of aerobic gram positive organism from different types of specimens			
	Respiratory specimen	Urine	Pus	Blood
linezolid	100	100	100	100
vancomycin	100	100	100	100
ciprofloxacin	50	53	37	NA
amikacin	54	NA	74	81
gentamicin	82	90	65	52
cefoxitin	72	88	65	76
clindamycin	56	NA	52	57
trimethoprim/ sulphamethoxazole	42	87	45	NA
nitrofurantoin	NA	43	NA	NA

Discussion:

The present study was undertaken in the department of microbiology to determine aerobic bacterial pathogen and their susceptibility pattern. This will help to understand the sensitivity pattern of various pathogens isolated and drug resistance in them. The antibiogram derived from the current evaluations will be effective antibiotic policy for the tertiary care hospital. SSTIs (n=1229, 60%) were the most common infections followed by LRTIs (n=471, 22.61%), UTIs (n=255, 12.24%) and BSIs (n=130, 6.23%). The increased rate of SSTIs may be due to inclusion of all types of wound infections (post surgical as well as primary skin infections). Similarly SSTIs were found as 72% in one study [10] and 78% in another study. [11] However, lower rate was reported by Shrestha and Basnet (50%). [12] We found the percentage of gram negative bacteria, gram positive bacteria as (n=339/1229) 28% and (n=379/1229) (31%) respectively. Past studies from India, showed that infections due to gram-positive bacteria are predominant in case of SSTIs. [13,14] We found the prevalence of *E. coli*, *Klebsiella* sp., *Pseudomonas aeruginosa* and *S. aureus* was significantly higher among SSTIs. This could be because majority of samples were obtained from inpatient department (IPD). Similar results were projected in annual report of Indian Council of Medical Research as gram negative bacterial predominance in SSTIs on antimicrobial resistance surveillance.[15,16] We found that GNB from the SSTIs had highest sensitivity to meropenem, gentamicin and tobramycin. We observed the moderate sensitivity to amikacin, piperacillin tazobactum, ceftazidime, amoxycilin-clavulanic acid and ciprofloxacin. These observations were similar to the study [11] where more than 50% of all GNBs were resistant to oral antibiotic ciprofloxacin. Similarly, the sensitivity was good for amikacin (>49%) and ciprofloxacin (44%). [12] The least resistance was observed for amikacin. The very high resistance was noted for ceftriaxone. The multidrug resistant (MDR) *E. coli* and *Klebsiella* spp. isolates were found to be predominant among deep seated SSTIs.[11] Recent studies in India have shown a rising trend with gram-negative bacteria as etiological agent of SSTIs. Due to multidrug resistance among gram-negative bacteria, it became difficult to treat the SSTIs due to change in prevalent etiology and its resistance trends.[10] We found that gram positive isolates were 100% sensitive to vancomycin and linezolid and had shown good sensitivity for amikacin and gentamicin. This was similar as observed in various studies. [10, 12] In our study, the gram positive bacteria were moderately sensitive to clindamycin, cotrimoxazole and ciprofloxacin. This is in contrast to the study by Ramakrishna MS et al. [10] The cumulative resistance rate of bacteria from hospitalized patients was higher in our study. Similarly, the higher proportion of antimicrobial resistance among inpatients has been reported. [11, 17] In our study, uropathogens contributed (n=255) 12.24% of the total bacterial isolates. This percentage is lower as compared to the findings (40%) in a study by Jadhav A.G. and Nilekar S.L. [18] The lower percentage may be due to the regional variation. We found the percentage of GNB and GPC from the total bacterial isolates as 80% and 20% respectively. This is exactly same as the observations in the study by Beyene G and Tsegaye W.[19] In our study the common microorganisms isolated were *E. coli* (n=94, 37%), *Klebsiella sp.* (n= 66, 26%), *Pseudomonas sp.* (n=32, 13%), *S. aureus* (n=27, 11%), *Citrobacter sp*. (n=16, 6%) and other species (n=20, 8%). This is in concordance with the study [18] where *E.coli* (n=742, 50%) followed by *Klebsiella sp*. (n=305, 21%), *Pseudomonas aeuroginosa* (n= 12%), *S. aureus* (n= 97, 7%) were isolated. The *E.coli* was the commonest uropathogen and *Klebsiella sp*. as second reported pathogen in many studies. [20, 21, 22, 23] In our study, many other GNB from enterobacteriaceae were isolated as a causative agent. This could be because the maximum numbers of samples were from hospitalized patients. The similar information was found in a literature. The relative frequency of the pathogens varies depending upon age, sex, catheterization, and hospitalization. [24] Due to the rapidly evolving adaptive strategies of bacteria, the etiology of UTI and antibiotic resistance profile of bacterial uropathogens have changed considerably over the past years, both in community and nosocomial infections.[25] In our study, the GNB had the maximum sensitivity (> 50% strains showing sensitive pattern) for the antibiotics meropenem, piperacillin tazobactum, nitrofurantoin while gentamicin, amikacin , amoxicillin-clavulanic acid, ciprofloxacin were found moderately sensitive. The antibiotics gentamicin and nitrofurantoin had low level of resistance for GNB. [19, 26] We also found low level of resistance for gentamicin and nitrofurantoin among GNB. The good sensitivity for piperacillin tazobactum (87%), gentamicin (75%) and nitrofurantoin (69%) was observed by Bitew A et al.[26] In our study, the maximum resistance was seen with the antibiotics amoxicillin, ampicillin and ciprofloxacin as noted in various studies.[19, 26] The GPC in our study were 100% sensitive to vancomycin and linezolid. The sensitivity with gentamicin, cotrimoxazole, and ciprofloxacin was good in our study. The sensitivity for vancomycin, linezolid and gentamicin (97%) was found high among the GPC as noted in study by Bitew A et al. [26] The least sensitivity was seen for the nitrofurantoin in our study. This is opposite to the findings by Bitew A et al. [26] The findings were similar for gentamicin by Beyene G and Tsegaye W. [19]

We found the bacterial isolates from LRTIs cases as (n=471) 22.61% among the total isolates (n=2083). This is close to the observations (n= 515, 14%) in the study by Jadhav AG and Nilekar SL.[18] We found GNB and GPC as 89% and 11% respectively. Similar findings were observed in our previous study [27] We didn't find any similarity to the observations as found in two different studies where the related findings were (GNB 78%, GPC 22%) and (GNB 76%, GPC 21%) respectively.[28, 29] In our study, the bacteria isolated were as *Klebsiella pneumoniae (44%), P. aeuroginosa (18%), S. aureus (11%), E.coli (9%), Acinatobacter sp. (8%), Citrobacter sp.* (6%) and others (4%). The most predominant single pathogen was *Klebsiella pneumoniae* (49.9%), *Escherichia coli* (13.3%), followed by *P. aeruginosa* (12.5%) was found in a study by Maduakor Uzoamaka et al. [29] The least number of *Staphylococcus aureus* (2.1%) were isolated in the same study [29] which is not in concordance with our observations. Similarly, the numbers of bacterial species such as *Klebsiella pneumoniae* (45.1%) followed by *Citrobacter freundii* (12.9%), *Pseudomonas aeruginosa* (9.6%), and *Staphylococcus aureus* (10%) were observed by K V Ramana et al. [30] The reason was inclusion of hospitalized patients in both these studies. Among the gram-positive bacteria, *Streptococcus pneumoniae* (n = 30, 51.7%) was the most predominant pathogen, followed by *Staphylococcus aureus* (n = 28, 48.3%) while *Pseudomonas aeruginosa* (n = 71, 35.32%) was the most predominant followed by *Haemophilus influenzae* (n = 68, 33.83%), *Klebsiella pneumoniae* (n = 36, 17.19%), and *Escherichia coli* (n = 26, 12.94%) among the GNBs.[28] The increased number of *Pseudomonas aeruginosa* and *A. baumannii* as a etiological agents is perfect example of opportunistic pathogen of human and is well known for nosocomial infection.[28] We have included bacterial isolates of *H. influenza* and *S. pneumoniae* in group "other" bacteria whose number is 4%. The Low prevalence of *H. influenzae* could be due to biofilms formation in vivo, which may yield negative cultures.[31, 32] Also, the evidence has indicated that *H. influenzae* is viable inside host cells, including macrophages and respiratory epithelial cells.[33] The lowest number of isolates of *S. pneumoniae* and *H. influenza* were found in our study. This may be due to the fact that these are the commonest causes of community acquired pneumonia. [34] In a systematic review, it has been mentioned that *Acinetobacter spp*. (31.68%), *P. aeruginosa* (16.59%), *H. influenzae* (14.30%), and *S. pneumoniae* (13.80%) were common isolates. The findings of same review indicated a three-fold increase was observed in the data of *A. baumannii* infections. *A. baumannii* was most frequently isolated during 2014 - 2020. [35] The differences observed in our findings may be because the distribution of LRTI etiological agents may vary depending on the geographical region, season, age, ethnicity, and underlying diseases. [36] In our study, among the GNB, the gentamicin, meropenem, piperacillin-tazobactum, ceftazidime had very good sensitivity (> 60% strains were sensitive). We observed the moderate sensitivity with amikacin, ciprofloxacin, tobramycin (approximately 50% strains were sensitive). Similar sensitivity pattern for *P. aeuroginosa* was observed by Khan S et al. [28] Among the GPC, 100% sensitivity was seen with vancomycin, linezolid. The sensitivity for gentamicin, amikacin, clindamycin and ciprofloxacin was more than 50%. Similar findings were observed in various studies. [27, 28] We found the positive blood culture as 6.23% which is less when compared to the study by Jadhav AG and Nilekar SL.[18] GPC and GNB as etiological agent were found in equal proportions. This is not true as in other studies where GNB predominates [37,39] but same in few studies.[38,41] We have found commonest causative agent as *S. aureus* (48%) and *K. pneumoniae* (22%) followed by *E. coli* (9%), *Acinatobacter sp*. (8%) *Citrobacter sp*. (6%) *P. aeuroginosa* (4%) and others (4%). In a study by Jadhav A G and Nilekar S L. [18] *S aureus* was the commonest cause of BSIs. In other study, the lesser percentage of *S. aureus* (6%), *K. pneumoniae* (5%) and more percentage of GNB such as *Acinatobacter sp*. (20%), *E. coli* (15%) was observed. The percentage of *P. aeuruginosa* was found same as (5%).[38] This is due to *Acinatobacter sp* is one of the commonest pathogen in nosocomial infections.[40] The percentage of *E. coli* (10%) and *Acinatobacter sp.* (6%) were isolated in a study by A.Vijaya Devi et al.[41] This is same as our findings. We had observed that meropenem and tobramycin had good sensitivity (60-62% strains were sensitive). The lower sensitivity was observed with the gentamicin, amikacin, piperacillin-tazobactum and ciprofloxacin in our study. This was in concordance with the findings of Khurana S at al. [39] The resistance pattern was similar as in the study by Jadhav A.G. and Nilekar S.L. [18] which mentioned that resistance to all classes of antimicrobials was high except colistin. Similarly imepenum and piperacillin-tazobactum were highly effective for GNB isolates. The lower sensitivity for three antibiotics gentamicin, amikacin and ciprofloxacin was observed by Vimala Venkatesh at al. [38] Imepenem and gentamicin showed good sensitivity for GNBs isolated from BSIs. [41] We found that all the GPC were sensitive to vancomycin and linezolid. The majority of GPC were sensitive to amikacin in our study. Similar findings were noted by Vimala Venkatesh at al. [38] The bacterial isolates of *S. aureus* were highly resistance to gentamicin and amikacin. [39] In our study, the prevalence of ESBLs and MRSA was (n= 629/1564) 40.21 % and (n= 189/519) 36.41 % respectively. The percentage of ESBL strains were observed more than 50% by El Aila et al. [42] It had been shown that nearly 40% urinary isolates of *E. coli* and *K. pneumoniae* were ESBL positive. [43] Many studies showed similar trend of MRSA from across India ranging from 26.14% to 43%. [44, 45] The factors responsible for rate of variations seen with different studies could be the different geographical area, variation in sample sizes and length of study, nature of specimens, methods used for testing, antibiotic policies, and status of infection control practices.

Conclusion:

In our study, the most common bacteria isolated were *Klebsiella species* followed by *Staphylococcus aureus*, *Pseudomonas species*, *Escherichia coli*, *Citrobacter species. Acinatobacter species* and others. The maximum sensitivity was observed for gentamicin and meropenem. The moderate sensitivity for piperacillin-tazobactum and tobramycin was found while low sensitivity to ceftazidime and fluroquinolones was noted among the GNB isolates. The amoxicillin clavulanic acid was the least sensitive antibiotic against Gram negative isolates. We had observed the better sensitivity for nitrofurantoin from urine isolates. The gram positive isolates were 100% sensitive to the antibiotics vancomycin and linezolid. The sensitivity to gentamicin, amikacin and clindamycin was good. Ciprofloxacin and cotrimoxazole showed least sensitivity. Nitrofurantoin had moderate sensitivity for GPC from urine isolates. Meropenem and vancomycin were the best drug combination for BSIs, LRTI, UTI and SSTIs as per antibiogram of aerobic bacteria isolated in our laboratory. The ESBL and MRSA strains were found as approximately 40% of GNB and GPC respectively. Thus the results of the current study emphasize the importance of institutional antibiotic policy for the effective and timely management of patients as there is increasing drug resistance profile of bacterial pathogen with regional variation.

Sources of supports: Nil
Conflicts of Interest: Nil

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