

Antibiotic sensitivity profile and resistance of microorganisms isolated from south Indian population, a hospital based study at Velappanchavady, Chennai, India

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ABSTRACT

Background: Increasing rates of antibiotic drug resistance has been noted in recent times and this adversely affects the prognosis and outcomes of patients. There is a greater need for local resistance prevalence data in order to guide empirical prescription and to identify areas in which medical need for newer antimicrobial agents is greater.

Methods: A prospective hospital based observational study was carried out to determine antibiotic sensitivity profile and resistance pattern of microorganisms. Samples were collected from urinary tract infections, while cultures from blood stream infections, sputum samples and Serology. Antibiotic susceptibility was determined by the standard disc diffusion method. Data interpretation was based on CLSI, 2017 guidelines for antimicrobial susceptibility testing.

Results: The predominant isolates from the samples were, *Staphylococcus aureus* (16.7%) 67, *K. pneumoniae* (11.5%) 46, *E. coli* (29.4%) 118, *P. aeruginosa* (6%) 24. *Escherichia coli*, the most common causative organism showed high resistance to commonly used drugs such as Ampicillin (60.1%) 71, Amoxicillin (53.4%) 63, Amoxicillin-clavulanic acid (44.1%) 52 and Nalidixic acid (53.4%) 63. *E. coli* was found to be most sensitive to Amikacin (51.7%) 61, Piperacillin (69.5%) 82, Norfloxacin (61.9%) 73, Meropenem (76.3%) 90 and Imipenem (68.6%) 81. *Klebsiella* was most sensitive to 30 (65.2%) ofloxacin, 31 (67.4%) ciprofloxacin followed by 24 (52.2%) ceftriaxone and least sensitive to 7 (15.2%) Amoxicillin and 12 (26.1%) Ampicillin.

Conclusions: Among commonly used antibiotics resistance to Penicillins (Ampicillin, Amoxicillin) was highest. Resistance to Fluoroquinolones (Ciprofloxacin) was seen in majority of the patients. Among broad spectrum antibiotics Imipenem, Meropenem resistance was seen in lesser proportion of the patients.

Keywords: Antibiotic sensitivity, Microorganisms, Resistance

INTRODUCTION

There is an increase in antibiotic resistance and a decline in new drug development. The primary reasons are widespread misuse of antibiotics, non-human antibiotic

use, poor quality of drugs, inadequate surveillance, poor healthcare standard, malnutrition, chronic and repeated infection, unaffordability of more effective and costly drugs. According to a 2014 report by World Health Organization (WHO) on global surveillance of

antimicrobial resistance, significant gaps in surveillance prevail, along with a lack of standards for methodology, data sharing and coordination. The additional problems are irrational antibiotic prescription by the physicians, habit of self-medication among patients, and indiscriminate use of antibiotics in agriculture and farming in different parts of the country.

To identify the present rate of resistance shown by the clinically significant pathogens, this study was conducted. Bloodstream infection is a major cause of morbidity and mortality despite the availability of potent antimicrobial therapy and advances in supportive therapy. Bacteremia due to gram negative bacilli pose serious therapeutic problems because of the increasing incidence of multidrug resistance.

Acute respiratory tract infections, such as bacterial pneumonia and acute exacerbations of chronic bronchitis, account for a considerable proportion of morbidity and antibiotic use. Moreover, these infections result in high mortality rates.¹ Unfortunately, the three major bacterial respiratory pathogens; *Streptococcus pneumoniae*, *Staphylococcus aureus* and *H. Influenza* have a worldwide increasing prevalence of antibiotic resistance.²⁻⁴

Gram negative bacteria were resistant to routinely used antibiotics, hence their resistant pattern should be considered essential before deciding the empirical treatment. The higher antibiotics should be reserved for multidrug resistant Gram-negative bacteria, whereas Linezolid and Vancomycin should be reserved for drug resistant Gram positive isolates.

The positive blood culture with antibiotic sensitivity of the isolated organism is the best guide to antimicrobial therapy, as resistance to antibiotics is a worldwide problem that causes ineffectiveness of empirical treatment. More so, strict infection control practices combined with judicious use of antibiotic therapy are the main solutions to this problem. However, it will be important to continue the surveillance of changes in trends and identify risk factors, to obtain information for empiric antibiotic therapy and to act rapidly in case of major changes in susceptibility patterns.

The recent misuse and overuse of antibiotics has induced changes in predominant bacterial species and their susceptibility to antibiotics, making it more difficult to treat. The importance of monitoring the progress of such resistance has led to numerous international, regional and national surveillance programmes. However, results from surveillance studies show wide variations in susceptibility rates, both geographically and over time.⁵ Prevalent flora and antimicrobial resistance pattern may vary from region to region depending upon the antibiotic pressure in that locality.⁶ Thus, there is a great need for local resistance prevalence data in order to guide empirical prescription and to identify areas in which medical need for new agents is greater.

METHODS

A prospective hospital based observational study was carried out to determine antibiotic sensitivity profile and resistance pattern of microorganisms. Samples were collected from urinary tract infection, while cultures from bloodstream infection, sputum samples and Serology.

Samples included sputum, for Gram stain and culture, blood samples for blood cultures and serum sample for serology. The valid sputum originating from the lower respiratory tract was defined as that containing squamous epithelial cell less than 10/high power field and polymorphonucleocytes more than 25/high power field. The organisms were isolated from sputum culture and blood culture. Serology was positive in all of cases. Indian studies showed sputum culture positivity is low in patients.⁷⁻⁹ Decreased sputum positivity is due to (a) inability of patients to expectorate due to altered sensorium because of severe disease (b) prior administration of antibiotics (c) 10-30% of patients have non productive cough. Blood cultures are valuable when positive but negative results are more common even in severe pneumonia. Positive blood culture results were observed in only few of patients with pneumonia.^{10,11}

Urine samples collected were clean catch midstream urine samples collected into a wide mouthed sterile container and inoculated on MacConkey and Blood Agar media using calibrated platinum loop following standard bacteriological technique and incubated at 37°C overnight. Pure bacterial colony counting 100,000 or more was considered as significant and was subjected to identification based on colony characters and biochemical tests. Blood culture was done after collecting the blood with aseptic precautions before starting antibiotics and 2 ml of blood was added to two bottles containing 25 ml of Brain heart infusion broth (HiMedia, Mumbai, India). Both the bottles were incubated aerobically at 37°C for 7 days. Subculture was done on sheep blood agar and MacConkey agar (HiMedia, Mumbai, India) routinely. Subculture was also done in between if visible turbidity appeared. The isolates were identified based on standard bacteriological techniques.¹² The growth of an organism was considered pathogenic if the same organism was isolated from both broths and contaminated if either the growth was obtained in only one bottle or a mixed growth was obtained.

Antibiotic sensitivity test was performed by disc diffusion method (Kirby-Bauer's technique) using commercially available discs (HiMedia, India) and the results were recorded following the instruction of the manufacturer. Antibiotic susceptibility was determined by the standard disc diffusion method. The antibiotic disks and their concentrations per disk (µg) comprised: Ampicillin (10), Cefotaxime (30), Gentamicin (10), Amikacin (30), Ciprofloxacin (5), Vancomycin (30), Piperacillin (100), Meropenem (10), Ceftriaxone (30), Ceftazidime (30), Amoxicillin (30), Erythro-mycin (15), Cefoperazone (75),

Cefoxitin (30), Colistin (10), Linezolid (30), Cefpodoxime (10). Data interpretation was based on Clinical and Laboratory Standards Institute guidelines for antimicrobial susceptibility testing (CLSI, 2017). Statistical analysis was carried out using the SPSS software (Version 17). Intergroup comparisons were performed with Chi-square test. A p-value of 0.05 or less was considered statistically significant.

RESULTS

A total of 401 samples positive for pathogenic organisms were collected. The age and sex distribution of patients was, 215 male patients and 186 female patients within the age group of 24 to 45 years. The most common organism isolated from blood culture was *Pseudomonas aeruginosa* followed by *Staphylococcus aureus* and *Streptococcus pneumoniae* and those from the sputum culture were *Streptococcus pneumoniae* followed by *Pseudomonas aeruginosa*.

The predominant isolates in patients were, *Staphylococcus aureus* (16.7%) 67, *K. pneumoniae* (11.5%) 46, *E. coli* (29.4%) 118, *P. aeruginosa* (6%) 24 (Table 1).

Staphylococcus aureus showed highest resistance to Penicillins like Ampicillin, Amoxicillin and least resistance to Imipenem and Meropenem (Table 2).

Escherichia coli, the most common causative organism of urinary tract infections showed high resistance to commonly used drugs such as Ampicillin (60.1%) 71, Amoxicillin (53.4%) 63, Amoxicillin-clavulanic acid (44.1%) 52 and Nalidixic acid (53.4%) 63. *E. coli* was found to be most sensitive to Amikacin (51.7%) 61, Piperacillin (69.5%) 82, Norfloxacin (61.9%) 73, Meropenem (76.3%) 90 and Imipenem (68.6%) 81 (Table 3). Gram negative bacterial isolates were more than Gram positive isolates in this study.

Table 1: Pathogens obtained from urine samples, blood culture, Sputum Culture and Serology.

Pathogen	Number	Percentage (%)
<i>E. coli</i>	118	29.4
<i>Klebsiella</i>	46	11.5
<i>Proteus</i>	23	5.7
<i>Staphylococcus Aureus</i>	67	16.7
<i>Streptococcus pneumoniae</i>	41	10.2
<i>Salmonella typhi</i>	28	7
<i>Enterococcus</i>	17	4.3
<i>Pseudomonas</i>	24	6
<i>Citrobacter</i>	12	3
<i>Acinetobacter</i>	14	3.5
<i>Enterobacter</i>	11	2.7

Klebsiella was most sensitive to 30 (65.2%) ofloxacin, 31 (67.4%) ciprofloxacin followed by 24 (52.2%) ceftriaxone

and least sensitive to 7 (15.2%) Amoxicillin and 12 (26.1%) Ampicillin (Table 4). Cotrimoxazole was ineffective against many *Pseudomonas* isolates and isolates of *Enterococcus spp.* Ciprofloxacin resistance was displayed by isolates of *Enterococcus* and *Pseudomonas*. *Pseudomonas* were sensitive to Meropenem 17(70.8 %), Imipenem 15 (62.5%), Piperacillin 13 (54.2%) and Amikacin 12 (50%) but showed higher degree of resistance to 8 (33.3%) Ciprofloxacin, 6 (25%) Gentamicin, 7 (29.2%) Cefotaxime and 5 (20.8%) Ceftriaxone (Table 5).

Table 2: Resistance and sensitivity of *Staphylococcus aureus*.

Antibiotics	Sensitive	Intermediate	Resistant
Amoxicillin	17(25.4%)	12(18%)	38(56.6%)
Ampicillin	18(26.9%)	9(13.4%)	40(59.7%)
Amoxicillin-clavulanicacid	23(34.3%)	15(22.4%)	29(43.3%)
Ciprofloxacin	41(61.2%)	11(16.4%)	15(22.4%)
Amikacin	28(41.8%)	12(17.9%)	27(40.3%)
Erythromycin	19(28.3%)	20(29.9%)	28(41.8%)
Cotrimoxazole	22(32.8%)	14(20.9%)	31(46.3%)
Ceftriaxone	36(53.8%)	8(11.9%)	23(34.3%)
Cefotaxime	39(58.2%)	11(16.4%)	17(25.4%)
Piperacillin	48(71.6%)	9(13.4%)	10(15%)
Meropenem	51(76.1%)	3(4.5%)	13(19.4%)

Table 3: Resistance and sensitivity of *E. coli*.

Antibiotics	Sensitive	Intermediate	Resistant
Amoxicillin	21(17.8%)	34(28.8%)	63(53.4%)
Ampicillin	18(15.3%)	29(24.6%)	71(60.1%)
Amoxicillin-clavulanicacid	30(25.4%)	36(30.5%)	52(44.1%)
Ciprofloxacin	69(58.5%)	26(22%)	23(19.5%)
Norfloxacin	73(61.9%)	21(17.8%)	24(20.3%)
Gentamicin	58(49.2%)	19(16.1%)	41(34.7%)
Amikacin	61(51.7%)	23(19.5%)	34(28.8%)
Erythromycin	71(60.2%)	19(16.1%)	28(23.7%)
Nalidixic acid	38(32.2%)	17(14.4%)	63(53.4%)
Nitrofurantoin	60(50.8%)	22(18.6%)	36(30.6%)
Cotrimoxazole	51(43.2%)	11(9.3 %)	56(47.5%)
Ceftriaxone	48(40.7%)	26(22 %)	44(37.3%)
Cefotaxime	53(45 %)	19(16.1 %)	46(38.9%)
Piperacillin	82(69.5%)	14(11.9 %)	22(18.6%)
Meropenem	90(76.3%)	11(9.3 %)	17(14.4%)
Imipenem	81(68.6%)	18(15.3 %)	19(16.1%)

In this study, *Pseudomonas aeruginosa* isolates were found to be highly resistant to first line antibiotics, followed by *Klebsiella pneumoniae* and *Escherichia coli*. The Gram-positive isolates were having better susceptibility to Amikacin, Cephalosporins and Ciprofloxacin; but were more resistant to Ampicillin and Gentamicin in the present study.

Table 4: Resistance and sensitivity of *Klebsiella*.

Antibiotics	Sensitive	Intermediate	Resistant
Ciprofloxacin	31(67.4%)	7(15.2%)	8(17.4%)
Ofloxacin	30(65.2%)	6(13%)	10(21.8%)
Amoxicillin	7(15.2%)	14(30.4%)	25(54.4%)
Ampicillin	12(26.1%)	9(19.6%)	25(54.3%)
Amoxicillin-clavulanic acid	6(13%)	16(34.8%)	24(52.2%)
Gentamicin	24(52.2%)	11(23.9%)	11(23.9%)
Amikacin	28(60.8%)	8(17.4%)	10(21.8%)
Erythromycin	24(52.2%)	9(19.6%)	13(28.2%)
Cotrimoxazole	16(34.8%)	7(15.2%)	23(50%)
Ceftriaxone	24(52.2%)	7(15.2%)	15(32.6%)
Cefotaxime	24(52.2%)	6(13%)	16(34.8%)

Table 5: Resistance and sensitivity of *Pseudomonas*.

Antibiotics	Sensitive	Intermediate	Resistant
Ciprofloxacin	8(33.3%)	2(8.3%)	14(58.3%)
Gentamicin	6(25%)	3(12.5%)	15(62.5%)
Amikacin	12(50%)	4(16.7%)	8(33.3%)
Ceftriaxone	5(20.8%)	2(8.3%)	17(70.8%)
Cefotaxime	7(29.2%)	5(20.8%)	12(50%)
Piperacillin	13(54.2%)	1(4.2%)	10(41.6%)
Meropenem	17(70.8%)	3(12.5%)	4(16.7%)
Imipenem	15(62.5%)	4(16.7%)	5(20.8%)

DISCUSSION

The antibiotic sensitivity pattern of organisms changes rapidly over a short period. It is especially true for developing countries where antibiotics are prescribed irrationally not only by the medical practitioners but the antibiotics are also purchased directly from the chemists without prescription. Reliable statistics on antibiotic resistance that are mandatory to control spread of resistant pathogens are available from the developed nations. These data are generated by large surveillance studies in countries such as the USA, Europe, Australia. However such data are sparse in developing countries like India due to the lack of large scale studies. Hospital antibiograms are commonly used to help guide empiric antimicrobial therapy and are an important component of detecting and monitoring trends in antimicrobial resistance. According to Louie et al staphylococci were identified by standard methods including the gram stain, catalase test and tube coagulase test.¹³

In contrast to the results of our study where gram negative organisms are more predominant, in developed countries Gram positive bacteria are more commonly reported. This was in concordance with Aletayeb SMH et al, and Sundaram V et al.^{14,15} The present study shows increased resistance to Ampicillin, Amoxicillin, Cotrimoxazole and Ceftriaxone, these findings are in agreement with the increasing prevalence of resistance to these antimicrobial agents demonstrated by Egyptian, regional, and world-

wide studies.^{16,17} The increased resistance to *Pseudomonas* could be due to increased use of antibiotics.^{18,19} *S. aureus*, *E. coli* and *Pseudomonas* are the major pathogens in the present study similar to studies from other parts of India and worldwide.²⁰⁻²²

The bacteriological profile for causative organisms differs significantly between developed and developing countries.^{23,24} *Klebsiella pneumoniae* is the most common bacterial agent causing sepsis in developing countries, while *Streptococcus* and staphylococci are the common agents in developed countries.²⁵⁻²⁷ Even among developing countries, regional variation in prevalence of the bacterial agents causing infections exists.^{28,29}

The overall improvement in the survival rate due to newer drugs, better care and advanced life support facilities has led to a change in the spectrum of agents causing sepsis in developed countries. However, there is a paucity of data on the recent trends of organisms causing sepsis in developing countries. Indian studies over the last three decades have reported high incidence of gram negative organisms among culture positive cases.³⁰⁻³³ *Klebsiella* and other Gram-negative organisms were the common causes of infection in the present study as well other studies from India and Nigeria. Hence there is importance to prevent infection by *Klebsiella pneumoniae*. Amikacin should be used along with third generation cephalosporins for empirical treatment of gram negative sepsis.

In a study from North India, 30–80% of the Gram negative isolates were resistant to third generation cephalosporins. Frequent local treatment during repetitive infections also causes the spread of resistant strains from hospital to patients and vice versa.³⁴ The occurrence of *P.aeruginosa* as the predominant offending organism could be attributed to its minimum nutritional requirements and its relative resistance to antibiotics.³⁵ In the present study, prevalence of *Staphylococcus aureus* (16.7%) is similar to that reported from Nagpur (19.56%) and Vellore (24%) in India.^{36,37} Finding of more concern is the resistance of positive isolates to piperacillin-tazobactam (beta lactam-beta lactamase inhibitor) combination since they are the antibiotics of choice in the treatment of infection due to resistant bacteria or the carbapenems.³⁸

A first line antibiotic treatment should be primarily directed against the pathogen. For coverage of gram negative bacteria, beta lactam-beta lactamase inhibitor combinations would be more useful. Use of mono drug therapy with cephalosporins, aminoglycosides and fluoroquinolones need to be guided by the sensitivity report. Lastly, continued monitoring of susceptibility pattern need to be carried out in individual settings so as to detect the true burden of antibiotic resistance in organisms and prevent their further emergence by judicious use of drugs.

In most clinical situations, there is a need to initiate empirical antimicrobial therapy before obtaining the

microbial results. However, the situation is further complicated by the emergence of MDR pathogens. Obviously, there is a great need for obtaining data on prevalent strains; along with the susceptibility pattern, to help in revising antibiotic policy and guiding clinicians for the better management of patients; particularly in developing countries. This "local" pattern of predominance should be taken into consideration upon prescribing antimicrobials in this locality. Obviously, this "local" difference explains the changing pattern of causative pathogens over time, even at the same hospital. Fortunately, this higher prevalence was closely related to the susceptibility pattern. This confirms the importance of implementing continued local surveillance programmes.

Present findings together with previous ones are suggestive of need of periodic monitoring of antibiotic sensitivity pattern of the bacterial isolates to provide effective treatment and prevent the emergence of resistance among commonly used antimicrobial agents particularly in developing countries like ours. If the antibiotic according to the sensitivity pattern is administered to these patients at an early stage of the disease, morbidity and mortality due to microorganisms can be minimized. This can be tackled by multicentre large scale studies of antibiotic sensitivity pattern, to generate nationwide or more appropriately region-specific antibiograms.

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