Original Research Article

Antibiotic susceptibility pattern of bacteria isolated from patients of respiratory tract infection in a tertiary care hospital of Central India

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ABSTRACT

Background: Respiratory tract infections are one of the commonest types of infections affecting the Indian population and are a significant cause of morbidity and mortality. To deal with multidrug resistance one should be armed with region specific data about the susceptibility patterns of antimicrobials. This study was planned to identify the common pathogens responsible for the respiratory tract infection and their antibiotic susceptibility patterns.

Methods: Smears were prepared from the specimens and gram stains of all clinical samples were done. The organisms were grown on various media and identified according to the standard procedures. The susceptibility patterns of the bacterial pathogens were determined following the panel of antimicrobial agents. Descriptive statistics were used for data analysis.

Results: Out of 513 samples 471 samples were further processed. Klebsiella (30.42%) was found to be commonest pathogen followed by Pseudomonas (28.36%). Ampicillin and piperacillin /tazobactam combination were found to be highly efficient against Pseudomonas (83% and 58.8% respectively), E. coli (68% and 50.7% respectively), and Klebsiella (43% and 30.3% respectively).

Conclusions: In our study, we found penicillins as the most efficacious antimicrobials to treat respiratory pathogens. The study reveals that the antibiotic susceptibility pattern varies across regions. The prescription of a right antibiotic, for a right patient will help to minimize the menace of emergence of antimicrobial resistance.

Keywords: Drug resistance, Respiratory tract infections, Susceptibility pattern

INTRODUCTION

Respiratory tract infections are one of the commonest types of infections affecting the Indian population with prevalence rates ranging around 52%.¹ Respiratory infections, particularly those affecting the lower respiratory tract, are significant causes of morbidity and mortality.² The majority of antibiotics prescribed in general practice are for respiratory tract infections (RTIs).³ ⁴ The incidence, etiology and prognosis vary depending on age, concurrent illness and predisposing risk factors as well as on whether such infections are community or hospital acquired.³ ⁸ To add to the trouble, statistical data and evidences from researches prove that multidrug resistance bacteria are rapidly emerging across the world and pose a big challenge to health care. Multidrug resistant bacteria cause serious nosocomial and community acquired infections that are hard to eradicate using available antibiotics. Extensive and non-specific use of broad spectrum antibiotics in hospitalized patients has led to both increased carriage and the development of multidrug - resistant strains that produce extended - spectrum beta-lactamase (ESBL).³ ⁹

Although the antimicrobial resistance has increased over a period of time, the development of novel antimicrobial agents has dramatically declined over the past 30 years.¹⁰
Therefore, to prevent the return of the pre-antibiotic era, one must use existing antibiotics skillfully and more judiciously. It’s difficult to manage these infections effectively unless we are armed with adequate and good quality data about the antibiotics susceptibility patterns of organisms causing respiratory tract infections. To be effective, this data has to be region specific and also has to be updated regularly as bacterial susceptibility varies across time and space. Unfortunately, such data is scant and this has led to a wide spread morbidity and mortality due to respiratory infections in central India. Hence, the study was planned to identify the common pathogens responsible for the respiratory tract infection and their antibiotic susceptibility patterns.

METHODS

The present study was conducted at Sri Aurobindo Institute of Medical Sciences, Indore. The participants of the study were all the patients of lower respiratory tract infection admitted in the Intensive Care Unit of Hospital during January 2015 to December 2016. After the due written informed consent from the participants or their guardian, the Lower Respiratory Tract (LRT) samples such as sputum, suction tip, endotracheal aspirate, bronchial aspirate and pleural fluid were obtained under aseptic precautions and were processed immediately. Macroscopic appearance of the sample was noted for color, consistency, quantity, and for presence of any pus or blood. Smears were prepared from the specimens and gram stains of all clinical samples were examined under compound microscope using oil immersion objective lens. The smear was examined for presence of pus cells, lymphocytes and epithelial cells. Gram reaction and arrangement of bacteria was seen.

Each sample was inoculated on blood agar and MacConkeys agar and incubated at 37°C for 24 hours and further for 48 hours if necessary, the organisms grown were identified on basis of their morphology, cultural characters and biochemical reactions according to standard procedures. Isolates from repeat culture of previously recruited patients and isolates identified as commensals or contaminants were excluded. The bacterial isolates were subjected to susceptibility testing by standard Kirby Bauer disc diffusion methods. The susceptibility patterns of the bacterial pathogens were determined following the panel of antimicrobial agents as recommended by Clinical Laboratory Standard Institute (CLSI) -2010.

Preparation of plates

Muller Hilton agar (Hi-Media) was prepared from a commercially available dehydrated base according to manufacturer’s instructions and was used for antimicrobial susceptibility testing. Representative sample of each batch of plates was examined for sterility by incubating at 35-37°C for 16-18 hours or longer. Before inoculation; plates were dried with lids ajar so that no moisture droplets were present on the agar surface.

Preparation of inoculums

At least 3-5 well-isolated, morphologically similar colonies from an agar medium were touched with a straight wire loop and growth was transferred to a test tube containing 4-5ml of sterile peptone water. The tubes were incubated for 2 to 6 hrs at 37°C to produce a bacterial suspension of moderate cloudiness.

Inoculation

Plates were inoculated within 15 minutes of preparation of suspension so that the density does not change. A sterile cotton swab was dipped into the suspension and surplus removed by rotation of the swab against the side of the tube above the fluid level. The medium was inoculated by even streaking of the swab over the entire surface of the plates in three directions.

Applications of antibiotic discs

Antibiotic discs were stored at 4°C in refrigerator. The discs were allowed to come to room temperature before application. After the inoculated plates were dried antibiotic discs were applied to the agar surface with a sterile forceps and gently pressed down to ensure contacts. They were distributed evenly so that they were not closer than 24mm from centre to centre. not more than 8 discs were applied on 90mm plate. Concentration of antibiotics used for testing susceptibility according to CLSI guidelines.

Zone diameter was measured in millimeters and interpreted as per CLSI guidelines. The identification of the bacterial isolates up to species level and their antimicrobial susceptibilities were also confirmed using Vitek 2 compact (BioMérieux, France). The entire testing was done under strict quality control and American Type Culture Collection (ATCC) strains were used as control strains.

RESULTS

Four hundred and twenty-three patients participated in the study and 513 samples were received in the microbiology department. Forty-two samples were excluded due to reasons such as contamination, inadequate quantity and poor labeling and the rest 471 samples were further processed. The samples obtained were from tracheal swab, endotracheal tube, pleural fluid, throat swab and sputum and their percentage distribution was as stated in Table 1.

The discriminative prevalence of various organisms across the sample site was assessed and Klebsiella (30.42%) was found to be commonest pathogen isolated from all the sites followed by Pseudomonas (28.36%) as
the next common pathogen isolated across all the sites. The discriminative prevalence of rest of the organisms in the decreasing order were Staphylococcus (14.89%), E. coli (10.93%), Acinetobacter (9.82%), Enterobacteriaceae (2.85%), Streptococcus (1.74%) and Enterobacter (0.95%). Klebsiella was the most prevalent organism isolated from Trachea (31.05%), Endotracheal (30.40%) and Sputum (32.08%) where as in pleural fluid sample Pseudomonas was the most prevalent (30.76%) and in throat swab Staphylococcus was the most prevalent (42.85%) organism isolated (Table 2).

Table 1: Number and percentage distribution of various samples.

| Type of organism | Tracheal | Endotracheal | Pleural fluid | Throat swab | Sputum | Total n (%) |
|------------------|----------|--------------|---------------|-------------|--------|-------------|
| Staphylococcus   | 34 (15.52%) | 23 (13.45%) | 03 (11.53%) | 12 (42.85%) | 22 (11.76%) | 94 (14.89%) |
| Streptococcus    | Nil | 01 (0.58%) | Nil | Nil | 10 (5.34%) | 11 (1.74%) |
| Pseudomonas      | 66 (30.13%) | 46 (26.90 %) | 08 (30.76%) | 06 (21.42%) | 53 (28.34%) | 179 (28.36%) |
| Klebsiella       | 68 (31.05%) | 52 (30.40 %) | 05 (19.23%) | 07 (25%) | 60 (32.08%) | 192 (30.42%) |
| Acinetobacter    | 21 (9.58%) | 32 (18.71 %) | 02 (7.69%) | Nil | 07 (3.74%) | 62 (9.82%) |
| E. coli          | 22 (10.04%) | 13 (7.60 %) | 06 (23.07%) | 03 (10.71%) | 25 (13.36%) | 69 (10.93%) |
| Enterobacter      | 02 (0.91%) | 02 (1.16%) | Nil | Nil | 02 (1.06%) | 6 (0.95%) |
| Enterobacteriace | 06 (2.73%) | 02 (1.16 %) | 02 (7.69%) | Nil | 08 (4.27%) | 18 (2.85%) |
| Total            | 219 | 171 | 26 | 28 | 187 | 631 |

Table 2: Discriminative prevalence of isolated organisms.

Table 3: Susceptibility patterns of pathogens causing respiratory tract infection.

| Type of organism | Staphylococcus (n=94) | Streptococcus (n=11) | Pseudomonas (n=179) | Klebsiella (n=192) | Acinetobacter (n=62) | E. coli (n=69) | Enterobacter (n=18) | Enterobacteriaceae (n=6) |
|------------------|-----------------------|----------------------|---------------------|-------------------|---------------------|----------------|---------------------|-------------------------|
| Penicillin group  |                       |                      |                     |                   |                     |                 |                     |                         |
| Penicillin-G      | 4.2                   | 9                    | ND                  | ND                | ND                  | ND             | ND                  | ND                      |
| Ampicillin        | ND                    | 9                    | 83                  | 43                | 17                  | 68             | 16.6                | 13.3                    |
| Piperacillin      | ND                    | ND                   | 39.4                | 7.3               | 3.2                 | 5.7            | 16.6                | 5.5                     |
| Ticarcillin       | ND                    | ND                   | 16.6                | 3.6               | 1.6                 | 1.4            | ND                  | 5.5                     |
| Oxacillin         | 13.8                  | ND                   | 1.6                 | 0.5               | 1.6                 | ND             | ND                  | ND                      |
| Amp/sulbct        | ND                    | ND                   | 0.5                 | 1.6               | 1.4                 | ND             | ND                  | 5.5                     |
| Pip/Tazo          | ND                    | ND                   | 58.8                | 30.3              | 14.5                | 50.7           | 16.6                | 22.2                    |
| Cephalosporin     |                       |                      |                     |                   |                     |                 |                     |                         |
| Cefazoline        | ND                    | ND                   | 0.5                 | 4.1               | ND                  | 8.6            | ND                  | ND                      |
| Cephalothin       | 2.1                   | ND                   | 0.5                 | 0.5               | ND                  | ND             | ND                  | ND                      |
| Cefoxin           | 14.8                  | ND                   | 2.7                 | 18.3              | 4.8                 | 18.8           | ND                  | 16.6                    |
| Cephotaxime       | ND                    | ND                   | 18.8                | 5.7               | 1.6                 | 10.1           | 16.6                | 5.5                     |
| Ceftazidime       | ND                    | ND                   | 24.4                | 5.2               | 1.6                 | 10.1           | ND                  | 5.5                     |
| Ceftriaxone       | ND                    | ND                   | 0.5                 | ND                | ND                  | ND             | ND                  | ND                      |
| Cefepime          | ND                    | ND                   | 22.2                | 5.2               | 3.2                 | 14.4           | 16.6                | 5.5                     |
| Monobactams, Carbapenems and Glycopeptides groups | | | | | | | | |
| Aztreonam         | ND                    | ND                   | ND                  | ND                | 1.6                 | 13             | 16.6                | ND                      |
| Imipenem          | ND                    | ND                   | 66.6                | 57.5              | 29                  | 78.2           | 83.3                | 50                      |
| Meropenem         | ND                    | ND                   | 41.1                | 29.3              | 27.4                | 36.2           | 66.6                | 22.2                    |
| Vancomycin        | 90.4                  | 100                  | 0.5                 | ND                | ND                  | ND             | ND                  | ND                      |

Penicillins, Cephalosporins, Monobactams, Carbapenems and Glycopeptides groups, ND = Not Done

Amongst the penicillin group of antibiotics, ampicillin and piperacillin/tazobactam combination were found to be highly efficient against Pseudomonas (83% and 58.8% respectively), E. coli (68% and 50.7% respectively), and Klebsiella (43% and 30.3% respectively). Pseudomonas was found to be more susceptible than other organisms for the penicillin group of antibiotics.
Most of the pathogens showed low susceptibility to cephalosporin’s, including fourth generation cephalosporin’s. Among all the cephalosporin’s, ceftazidime, a generation cephalosporin showed relatively higher efficacy against Pseudomonas (24.4%). Vancomycin showed higher activity against Streptococcus (100%), followed by Staphylococcus (90.4%). Whereas, imipenem exhibited its action against Enterobacter (83.3%), followed by E. coli (78.2) and Pseudomonas (66.6), meropenem was also found to be effective against Enterobacter (66.6%). However, aztreonam shows relatively lower susceptibility towards pathogens (Table 3).

Chloramphenicol and tetracycline were found to be highly effective against Staphylococcus (80.8% and 75.5 % respectively). Amikacin exhibited its activity against Pseudomonas (83.3%) and E. coli (68.1%). Gentamicin was highly active against Streptococcus (72.7%). However, streptomycin and tobramycin did not show significant activity against these pathogens. Most of the fluoroquinolones and sulfonamides tested in this study did not exhibit significant efficacy against the pathogens. Linezolid was found to be highly active against Staphylococcus (89.3%) and Streptococcus (81.8%) whereas colistin was noted to be efficacious against Enterobacteriaceae (72.7%), E. coli (62.3%), Pseudomonas (57.2%), Klebsiella (56%), and Acinetobacter (48.3%). Other antimicrobial agents like clindamicin, erythromycin, rifampicin and polymixin-B did not exhibit significant activity against any of the pathogens (Table 4).

**DISCUSSION**

Sputum samples were the commonest specimen received in the microbiology lab for respiratory infections and contributed to 34.6% of all the samples followed by the tracheal swabs, endotracheal tube sample, pleural fluid and throat swab. Sputum samples are probably the easiest to collect, as noted in previous studies wherein 75% of the adult patients were able to produce an adequate sample of sputum for microbiological evaluation. Along with the ease of collection, sputum also has a high sensitivity (75%) to detect the bacterial pathogens in case of a lower respiratory tract infection. The various organisms were isolated and cultured from these samples and their susceptibility testing was done according to Clinical and Laboratory Standards Institute Guidelines (CLSI) with the intention of assessing the discriminative prevalence of various common bacteria and to identify the local prevalent antibiotic response against the detected pathogens. Most commonly found bacteria in the present study were Klebsiella (30.42%) and Pseudomonas (28.36%) followed by Staphylococcus, Acinetobacter and E. coli. A very small number of specimens exhibited presence of Enterobacter (2.8%), Streptococcus (1.7%), and Enterobacteriaceae (0.9%). Syed Mustaq Ahmed et al, did a study to identify the bacteriogram of all the gram negative lower respiratory tract pathogens.
tract infections and they found *Klebsiella* (41.95%) to be the commonest bacteria causing the infection followed by *Pseudomonas* (26.84%). Similarly, Siddalingappa et al. also studied sensitivity pattern of bacteria causing respiratory tract infections in a hospital in southern India. Out of 466 culture positive samples, 51.1% were positive for *Klebsiella* and 19.1% were positive for *Pseudomonas*. The least prevalent bacterium was enterococci (1.3%). Sharma et al. tried to identify the common bacteria causing respiratory tract infection and its antibiotic susceptibility. A total of 173 individuals (94 males and 79 females) sputum specimens were tested for bacterial infection. *Klebsiella* was the commonest bacteria isolated 26.2% followed by *Streptococcus pneumonia*, *E. Coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. All these three Indian studies have shown *Klebsiella* to be the commonest isolated bacteria and the same was found in our study as well. *Pseudomonas* was the second most prevalent bacteria in our study and so was it in two of the three Indian studies discussed.

Researchers have frequently stressed on the rising resistance of organisms against penicillin group of antibiotics. In fact, Akingbade et al. reported that all the *Pseudomonas* (100%) and *E. coli* (100%) organisms in his study were resistant to ampicillin. In contrast to the popular findings, amongst the penicillin group of antibiotics, ampicillin showed significant activity against *Pseudomonas* (83%) followed by *E. coli* (68%) in our study. Ampicillin was also highly active against *Streptococcus* (90%) obtained from sputum sample. Such a contrast can probably be explained with the change in the sensitivity patterns across regions and time. Kaushik et al. had mentioned in his study that after decades of resistance of ampicillin, *Salmonella* is gradually getting sensitive to ampicillin again. Other organisms exhibited resistance towards most of the members of penicillin group of antibiotics. Combination of piperacillin/tazobactam showed significant action against *Enterobacteriaceae* (100%) and *E. coli* (83.3%) obtained from pleural fluid.

All the bacteria isolated from different specimens showed resistance against the cephalosporin group of antibiotics. Relatively, cefazidime and cefepime were the better choices of treatment in pseudomonas infections (24.4% and 22.2%). Syed Mustaq Ahmed et al. also reported cefazidime to be a better choice amongst cephalosporin's for *Pseudomonas* infection (42.9%). *Klebsiella* showed high resistance to all the cephalosporin's, with the highest resistance to cephalothin (99.5%) and ceftriaxone (99.5%).

All the isolated bacteria low susceptibility towards aztreonam except pathogens like Enterobacter, *E. coli* and *Pseudomonas* showed relatively higher susceptibility (83.3%, 78.2% and 66.6% respectively) to imipenem. These findings match with the findings of Pathamanathan et al., who stated that 79.4% of *Pseudomonas* isolates were susceptible to imipenem. Imipenem also exhibited significant action against Enterobacter (100%), *E. coli* (72.7%), and *Pseudomonas* (63.6%) in tracheal sample. In pleural fluid imipenem was found to be effective against *Enterobacteriaceae* (100%), *E. coli* (100%), and *Klebsiella* (80%). Meropenem did not show significant activity against any of the bacteria in our study, highest activity being 66.6% against Enterobacter. This finding contradicts the findings of Urmia Jethwani et al., who reported 100% activity of meropenem against Enterobacter and *Pseudomonas*. Vancomycin in our study showed high efficacy against *Streptococcus* (100%) and *Staphylococcus* (90.4%) whereas *Klebsiella* exhibited high resistance towards it.

Broad spectrum antibiotics viz chloramphenicol and tetracycline exhibited relatively higher activity (80.8% and 75.5%) against *Staphylococcus* whereas all the other bacteria exhibited resistance against them. Such resistance against tetracycline and chloramphenicol has been frequently voiced in the past as well. Among the specimens of pleural fluid, tetracycline showed good activity against *Staphylococcus* (100%) and *Klebsiella* (100%) and so did chloramphenicol against *Staphylococcus* (100%). Even among throat swab isolates both chloramphenicol and tetracycline showed good activity against *Staphylococcus* (83.3% and 75% respectively).

Amikacin displayed relatively higher activity against *Pseudomonas* (83.3%). This finding is in consensus with the finding of Pathmanathan et al., who reported that 86.6% *Pseudomonas* were susceptible to amikacin. Gentamicin also demonstrated higher activity (72.7%) against *Streptococcus*. However, the respiratory pathogens exhibited resistance towards other aminoglycoside group of antibiotics.

Levofloxacin demonstrated lesser resistance towards majority of the isolates, although its highest activity being 50% was against Enterobacter. Similar results were reported by researchers earlier. Linezolid showed significant activity against *Staphylococcus* and *Streptococcus* (89.3% and 81.8% respectively). This finding is in consensus with the finding of Hackel M et al., who reported 100% sensitivity of linezolid against *Staphylococcus* and *Streptococcus*. Colistin showed some activity against *Enterobacteriaceae*, *E. coli*, *Pseudomonas*, *Klebsiella* and *Acinetobacter* (72.7%, 62.3%, 57.2%, 56% and 48.3% respectively). Such a resistance of colistin against *Pseudomonas* was also reported by Joseph et al. (67%).

**CONCLUSION**

In our study, we found penicillins as the most efficacious antimicrobials to treat respiratory pathogens. The study reveals that the antibiotic susceptibility pattern varies across regions and timeline and hence studies examining the local susceptibility patterns should be carried out at every centre. This will arm the clinician with complete
and updated information to select the apt antibiotic for the patients of respiratory tract infection and will reduce indiscriminate use of antimicrobials. This will help to prevent emergence of resistance strains of pathogens. The prescription of a right antibiotic, by a right doctor, for a right patient, in right doses, at the right time for right duration will be the best approach for minimizing the menace of emergence of antimicrobial resistance.

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