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Original Research Article

Incidence and sensitivity pattern of *Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa* in a tertiary care hospital

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ABSTRACT

Background: Antimicrobial resistance is a serious problem worldwide and differs from region to region. This study was planned to determine the incidence and sensitivity pattern of *Klebsiella pneumoniae (K. pneumoniae)*, *Escherichia coli (E. coli)* and *Pseudomonas aeruginosa (P. aeruginosa)* in our region and discuss the general issues related to antimicrobial resistance.
Methods: Prospective study was carried out between March to October 2015. Samples of urine, blood, pus, CSF and miscellaneous samples (fluids, swabs, sputum and stool) were collected from indoor and outdoor patients for isolation and antimicrobial susceptibility of *K. pneumoniae*, *E. coli* and *P. aeruginosa* in the Department of Microbiology G.R. Medical College, Gwalior (MP).
Results: Out of the 5000 samples analyzed 1684 showed growth. *K. pneumoniae* (38.50%), *E. coli* (33.29%) and *P. aeruginosa* (28.19%) constituited a total of 805 isolates. Both *E.coli* and *K. pneumoniae* showed highest sensitivity for doxycycline (75%; 67% resp.) and second highest for levofloxacin (70%; 64% resp.), whereas, *P. aeruginosa* showed highest 57% sensitivity for amikacin followed by 48% for levofloxacin. β-lactam antibiotics and aminoglycosides showed high mean resistance (*K.pneumoniae*-83%, *E.coli*-79%, *P. aeruginosa*-86.4%) and (*K. pneumoniae*-75%, *E. coli*-61%, *P. aeruginosa*-70%) resp.
Conclusions: The data indicates high resistance among the gram-negative bacteria for β-lactam and aminoglycoside antibiotics. Increasing resistance to doxycycline and flouroquinolones for *K. pneumoniae* and *E. coli* and multidrug resistance to *P. aeruginosa* is a cause of concern in this region. Thus, there is a need to stop misuse of antibiotics with immediate effect and to implement a strong antimicrobial stewardship program.

Keywords: Antimicrobial sensitivity, Gram-negative bacteria, Resistance, Stewardship

INTRODUCTION

In developing countries although many communicable diseases have been effectively contained, bacterial infections remain a major cause of morbidity and mortality. The discovery of antimicrobial agents had a major impact on the rate of survival from infections. Antimicrobial resistance nowadays is a well-known clinical and public health problem.[1] This is an emerging problem, especially in hospitals of the newly industrialized countries of Asia and the Pacific.[2]

Gram-negative bacteria cause infections including pneumonia, bloodstream infections, wound or surgical site infections and meningitis in healthcare settings. Gram-negative bacteria are resistant to multiple drugs and are increasingly becoming resistant to most available antibiotics. These bacteria have built-in abilities to find new ways to be resistant and can pass along genetic materials that allow other bacteria to become drug-resistant as well. Gram-negative infections include those caused by *Klebsiella pneumonia (K.pneumoniae)*, *Pseudomonas aeruginosa (P.aeruginosa)*, *Escherichia coli (E.coli)*, *Acinetobacter* as well as many other less common bacteria.

K.pneumoniae is ubiquitously present and reported worldwide. Antimicrobials effective against

K.pneumoniae are cotrimoxazole, flouraquinolones, piperacillin, amoxy-clav, third generation cephalosporins and aminoglycosides.[3] However, multidrug resistant bacteria are emerging causing serious nosocomial and community acquired infections that are hard to eradicate using available antibiotics.

E.coli, the most prevalent facultative Gram-negative bacillus in the human fecal flora, usually inhabits the colon as an innocuous commensal. Theoretically the antimicrobials effective against *E.coli* are cotrimoxazole, flouroquinolones, piperacillin, amoxy-clav, third generation cephalosporins, carbapenems and aminoglycosides.[3] Surveillance data show that resistance in *E. coli* is consistently highest for antimicrobial agents that have been in use the longest time in human and veterinary medicine.[4]

Known for many years to be a cause of serious wound and surgical infections, but often regarded as a secondary or opportunistic invader rather than a cause of primary infection in healthy tissues, *P.aeruginosa* has now clearly emerged as a major nosocomial pathogen in immunocompromised and debilitated patients, as well as in cystic fibrosis patients.[5] The antimicrobials known to be effective against *P.aeruginosa* are flouroquinolones (moderate efficacy), carbenicillin, piperacillin, ceftazidime and cefoperazone, carbapenems and aminoglycosides.[3] *P.aeruginosa* has the ability to resist (intrinsically or after acquisition of the necessary genes) a large number of antimicrobial agents, therefore, has always been considered to be a difficult target for antimicrobial chemotherapy.

The majority of bacterial infections are treated on the basis of a presumptive etiological diagnosis determined by the clinical history and physical findings. Empirical therapy should be based on local epidemiological data on likely pathogens and their patterns of antimicrobial susceptibility. Surveillance studies are valuable tool for assessing the changes in pattern of resistance of clinical isolates of antimicrobial agents and therefore, we planned this study to determine the sensitivity pattern of *E.coli*, *K.pneumoniae* and *P.aeruginosa* in our region.

METHODS

Sample collection

Samples of urine, blood, pus, CSF and miscellaneous (which included ascitic, pleural and peritoneal fluid; ear, throat and vaginal swab; sputum and stool) were collected from indoor and outdoor patients of various departments and hospital units for isolation and antimicrobial susceptibility pattern of *E.coli*, *K.pneumoniae* and *P.aeruginosa* in the department of Microbiology, G. R. Medical College, Gwalior, (M.P.) from March 2015 to October 2015, and these were analyzed and reported.

Identification of bacteria

The bacteria were cultured on MacConkey's agar, Nutrient agar, blood agar and other selective media followed by the identification of the isolates based on their cultural characteristics, gram staining, motility and reactions in standard biochemical tests.

Antimicrobial agents

All the isolates were tested for antimicrobial susceptibility by the Kirby-Bauer disk diffusion technique on Muller Hinton Agar by Filter Paper disks impregnated with antibiotics (Span diagnostics limited, Surat, India). A pre-diffusion time of 30 min was allowed at room temperature and the plates were incubated at 37°C for 24 h. The diameter of the zone of inhibition was measured and compared to that of standard strain and the results were interpreted as sensitive, or resistant, based on Clinical Laboratory Standard Institute 2014 guidelines.[6]

The percentage antimicrobial susceptibility of the isolated microorganism against different antimicrobials tested was calculated and interpreted as sensitive and resistant.

All the varied types of samples that came in the department of microbiology during the period of 8 months study were included.

The study was conducted after an approval from the Institutional medical ethical committee.

RESULTS

Out of total 5000 samples 1684 (33.68%) showed growth in the culture media. The various organisms isolated were *S.aureus*, *E.coli*, *K.pneumoniae*, *P.aeruginosa* and several others (like *acinetobacter*, *enterococci*, *streptococcus*, *proteus*, *citrobacter*, etc.). The rest of the samples i.e. 66.32% depicted no growth.

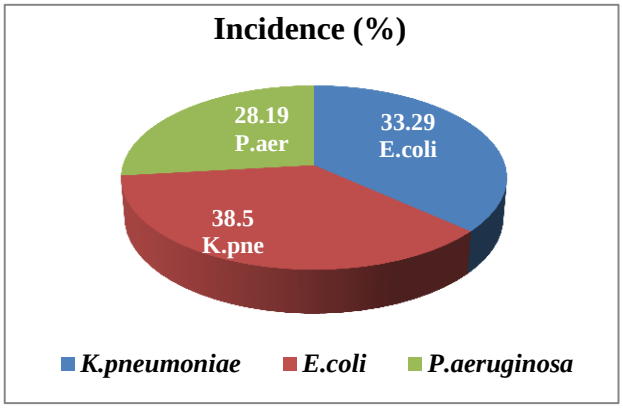


Figure 1: Incidence of three gram-negative *bacilli*.

A total of 805 bacterial isolates were comprised of *K.pneumoniae* (310 samples), *E.coli* (268 samples) and

P.aeruginosa (227 samples) with 38.50%, 33.29% and 28.19% incidence respectively (Figure 1).

Sensitivity pattern of K.pneumoniae, E.coli and P.aeruginosa for different antibiotics

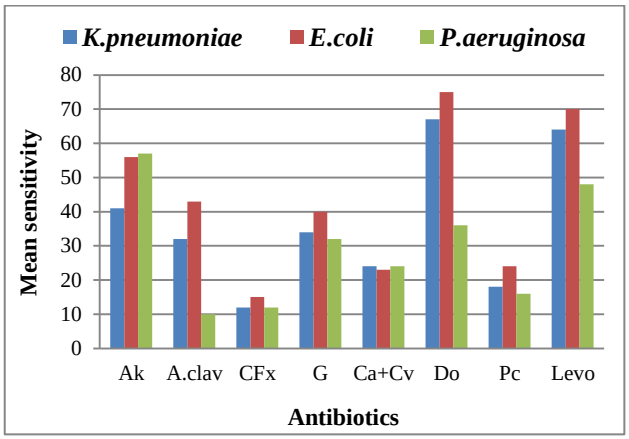
The mean sensitivity of *K.pneumoniae, E.coli* and *P.aeruginosa* for amikacin and gentamycin was found to be 41%, 56% and 57% and 34%, 40% and 32% respectively. In the penicillin group mean sensitivity was 32%, 43% and 10% and 18%, 24% and 16% for amoxy-clav and piperacillin for *K.pneumoniae, E.coli* and *P.aeruginosa* respectively. Cefixime showed 12%, 15% and 12% and ceftazidime + clavulinic acid showed 24%, 23% and 24% mean sensitivity respectively for *K.pneumoniae, E.coli* and *P.aeruginosa*. Mean sensitivity for doxycycline was 67%, 75% and 36% respectively for *K.pneumoniae, E.coli* and *P.aeruginosa*. Lastly levofloxacin showed 64%, 70% and 48% mean sensitivity for *K.pneumoniae, E.coli* and *P.aeruginosa* respectively (Figure 2).

The above mentioned antibiotics were exposed in good no. of samples to antibiotic susceptibility test and thus the result obtained from these can be relied upon.

Sensitivity pattern of K.pneumoniae, E.coli and P.aeruginosa to less exposed antibiotics

For *K.pneumoniae* the no. of samples tested were 48 (15%), 86 (28%) and 56 (18%) for ampicillin, fusidic acid and ofloxacin respectively. Likewise for *E.coli* it was 13 (5%), 159 (59%) and 67 (25%) for ampicillin, fusidic acid and ofloxacin respectively. Similarly for *P.aeruginosa* it was only 29 (13%), 61 (27%) and 62 (28%) for ampicillin, fusidic acid and ofloxacin respectively (Table 1).

The mean sensitivity for these antibiotics is depicted in Table 1. Since these antibiotics were exposed in few no. to antibiotic susceptibility test, their results are doubtful and much cannot be commented on.



Ak: amikacin, A.clav: amoxicillin+clavulinic acid, CFx: cefixime, G: gentamycin, Ca+Cv: ceftazidime+clavulinic acid, Do: doxycycline, Pc: piperacillin, Levo: levofloxacin.

Figure 2: Sensitivity pattern of *K.pneumoniae, E.coli* and *P.aeruginosa* for different antibiotics.

Table 1: Sensitivity pattern of *K.pneumoniae, E.coli* and *P.aeruginosa* to less exposed antibiotics.

Organism	Ampicillin		Fusidic acid		Ofloxacin	
	% of antibiotic exposed	Mean sensitivity (%)	% of antibiotic exposed	Mean sensitivity (%)	% of antibiotic exposed	Mean sensitivity (%)
K. pneumoniae	15	1	28	86	18	56
E. coli	5	0	59	62	25	43
P. aeruginosa	13	6	27	5	28	47

DISCUSSION

K. pneumoniae

Among the antibiotics tested *K.pneumoniae* showed maximum sensitivity for doxycycline (67%) followed by flouroquinolones (levofloxacin- 64%; ofloxacin- 56%). Rest of the antibiotics had less than 50% sensitivity i.e. aminoglycosides (amikacin- 41%; gentamycin- 34%), penicillin group 1-32% and third generation cephalosporins <30%. Our finding of sensitivity towards penicillin group was in accordance with other studies from different parts of India which showed not more than 40% sensitivity in any of them.[7-9] In case of cephalosporins some studies showed good sensitivity pattern while a study by Rakesh Asati supported our findings.[7-9] This increasing resistance for β-lactam group is probably due to production of ESBLs (extended spectrum β-lactamases). Perhaps most worrisome of the class A enzymes is the *KPC carbapenemase* that is rapidly emerging in the Enterobacteriaceae. This enzyme confers resistance to carbapenems, penicillins, and all of the extended-spectrum cephalosporins.[10]

We found <50% sensitivity for aminoglycosides but the other studies depicted 60-85% sensitivity for this group indicating that there is an increasing trend of resistance

for aminoglycosides in our region [7-9] Accquisition of cell membrane bound inactivating enzymes (by conjugation or transfer of plasmids) which phosphorylate/ adenylate or acetylate the antibiotic, which is then, incapable of enhancing active transport is the most common mechanism of resistance for aminoglycosides.[3]

Although our sensitivity for flouroquinolones was fairly good but not as good as shown by Manikandan et al.[9] This might show the possibility of resistance towards flouroquinolones which could be due to mutations in the bacterial chromosomal genes encoding DNA gyrase or topoisomerase IV or by active transport of the drug out of the bacteria.[10]

As mentioned above among all the antibiotics tested doxycycline emerged as most sensitive with 67% sensitivity. Rakesh Asati found 0 % sensitivity for tetracyclines.[7] A fair amount of sensitivity in our region could be due to less usage of this old antibiotic as compared to new ones like third generation cephalosporins, which got resistant due to over usage. Thus, these points out the fact that indiscriminate use of antibiotics leads to antimicrobial resistance.

E. coli

The sensitivity for penicillin antibiotics was <50% in our study. Some studies were in accordance with our results and some not.[11,12] The cephalosporins also failed to show good susceptibility against *E.coli* in our study [cefixime- 15% and ceftazidime+ clavulanic acid- 23%] that was in line with the other studies by Rakesh Asati and Urmi Jethani which also showed less than 30% susceptibility.[11,12]

The above results point out towards an increasing resistance of *E.coli* for the β-lactam group. The mechanism of resistance in gram negative bacteria for β-lactam is production of β-lactamases. Active efflux pumps serve as another mechanism of resistance, removing the antibiotic from its site of action before it can act.[10]

The aminoglycosides did not show promising results in our survey. But the other studies depicted better results indicating an increasing resistance towards this group in our region.[11,12] The mechanism of resistance of gram-negative bacteria for aminoglycosides is same as mentioned for *K.pneumoniae*.

However, our study showed doxycycline to be the best drug followed by levofloxacin. The other studies showed around 20-30% sensitivity for flouroquinolones and tetracyclines. We observed that older drugs like doxycycline is becoming less resistant now but newer drugs like cephalosporins are getting resistant. It indicates that routine exposure of bacteria only to newly developed antibiotics eliminated resistance against older out of use antibiotics and present bacterial strains have grown sensitive to these out dated agents.

P. aeruginosa

The conventional anti-pseudomonal agents are β-lactams (anti-pseudomonal penicillins and some of the third generation cephalosporins), aminoglycosides, flouroquinolones and carbapenems.

The maximum sensitivity for *P.aeruginosa* was shown by amikacin (57%) in our study, which is not a good percentage. A study by Mohan BS et al showed 67% sensitivity for amikacin and 72% for tobramycin.[13] The other two studies howsoever, supported our findings by showing sensitivity for aminoglycosides to be in the range of 30-50%[14,15] The sensitivity for gentamycin was consistently below 45% in all studies including ours.

Rest all the antibiotics were found to be resistant for *P.aeruginosa* in our study. The various studies from different parts of India however, depicted mixed results.[13-15]

The above scenario points towards the presence of multi drug resistant *P.aeruginosa* in our region as none of the usually effective antipseudomonal agents have shown good sensitivity. This is possible because *P.aeruginosa* shows a remarkable capacity to resist antibiotics, either intrinsically (because of constitutive expression of β-lactamases and efflux pumps, combined with low permeability of the outer-membrane) or following acquisition of resistance genes (e.g., genes for β-lactamases, or enzymes inactivating aminoglycosides or modifying their target), over-expression of efflux pumps, decreased expression of porins, or mutations in quinolone targets. Worryingly, these mechanisms are often present simultaneously, thereby conferring multi resistant phenotypes.[16]

Antimicrobial resistance poses many problems like the hospital stay of patient is increased, additive cost to the patient and community and thus, chances of infection with resistant organism to other patients increases. The various determinants of this problem are inappropriate and irrational use of medicines, inadequate and irregular supply of medicines in hospitals, use of broad spectrum antibiotics for empirical therapy, self-medication, poor compliance, poor infection prevention and control practices in hospitals and last but not the least a weak surveillance and regulatory system against antimicrobial resistance in the country.

The various measures which could be taken to curtail this problem is first and foremost to develop a strong stewardship program and antibiotic policy in each hospital so that drugs can be prescribed rationally according to the local pattern of sensitivity, prevent the misuse of drugs in other sectors like veterinary, pisciculture, agriculture, etc., strengthen communicable

diseases control programmes in country, create awareness in community and among different categories of health care professionals, develop standard infection control practices everywhere in country and most importantly establish a national alliance against antimicrobial resistance with all key stakeholders as its members and implement appropriate surveillance mechanisms in various sectors.

Limitations

It was done only for gram-negative bacteria in our hospital for a short period of time. Studies like this should be done on a regular basis covering all the organisms in every hospital and medical care centers so as to get updated and thus use the antibiotics rationally according to the local pattern of resistance.

CONCLUSION

To conclude, present study helped us to see the reality of antibiotic sensitivity pattern of bacteria in our region which is not good. And seeing the pace of new antibiotic development, immediate steps must be taken to curtail the problem of antibiotic resistance, otherwise it will take no time for us to move to pre-antibiotic era which would be a huge step back for human community.

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Ethical approval: The study was approved by the Institutional Medical Ethics Committee

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