Increasing emergence of antibacterial resistance mainly in uropathogens: southeast part of India

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Abstract- The existence of large concern over the increasing resistance to antibiotics for clinically important pathogens which cause varied number of diseases has led to monitor the prevailing resistance pattern of microbes in a study which was carried out in the city of Vijayawada in Andhra Pradesh. In this study, the data of urinary isolates which showed antibiotic resistance patterns were collected from government hospital, multispecialty hospital and a private laboratory. Total 1424 retrospective samples were collected and evaluated for antimicrobial resistance. The samples 676 (47.5%) were positive and 748 (52.5%) were negative to the infection. The antibiotics which showed higher rate of resistance by the urinary isolates were Amoxicillin, Ciprofloxacin, Co-Trimoxazole, Norfloxacin, Nalidixic acid. In contrast, organisms showed susceptibility to Amikacin and then to Azithromycin. Among the gram negative organisms, Escherichia coli was resistance to Amoxicillin (100%), Nalidixic Acid (92.6%), while Klebsiella pneumoniae was found resistance to Amoxicillin (96.3%), Gentamycin (83.3%), Pseudomonas aeruginosa was highly resistance (100%) to Amoxicillin, Ciprofloxacin, Norfloxacin, Nalidixic acid and Co-trimoxazole. Under gram positive organisms Staphylococcus aureus showed resistance to Amoxicillin (72.7%), Nalidixic acid (66.7%) and Enterococcus faecalis was resistance to Nalidixic acid (88.9%) and Norfloxacin (83.3%).
Key Words: antibiotics, resistance, biological samples, gram negative and gram positive organisms.

Introduction

Antibiotics are the important medicaments which are used to kill/inhibit the growth of micro-organisms which cause many infections (pneumonia, tuberculosis, gonorrhea, throat infections, typhoid etc.,) by their metabolic activities in human beings. Though the antibiotics are important, their inappropriate and large usage is causing the organisms to develop resistance towards the antibiotics, leading to delayed treatment to infections. So, this makes finding emerging antibiotics to fight against old diseases (because resistant strains of bacteria have emerged for resistance towards primary generation of antibiotics) and a parallel problem to find out next generation of antibiotics to fight against new diseases [1]. Antibiotic resistance occurs when an antibiotic has lost its ability to kill bacterial growth, in other words, the bacteria are resistant and continue to multiply in the presence of therapeutic levels of an antibiotic. The emergence and increasing pattern of antimicrobial resistance became a challenge to public health [2]. Poverty, inadequate access to drugs, increased use and misuse are major forces in the development of resistance [3]. Despite the plenty of progress in the antimicrobial chemotherapy, bacterial pathogens gradually develop resistance. Even the use of drug combinations has resulted in making microbial resistance to antibiotics. The first bacterium to show resistance was "Staphylococcus aureus", which causes pneumonia. It becomes resistant to penicillin within four years of its use by altering its cell wall. Haphazard use of antibiotics may also make the bacteria resistance to them [4].

Materials and Methods

This is a retrospective survey study of culture sensitive records for a period of one year at Government Hospital Laboratory (GHL), Multispeciality Hospital Laboratory (MHL) and a Private pathological Laboratory (PL). The culture sensitivity tests were performed by Kirby Bauer method by using antibiotic discs. A total of 1424 samples records were collected. Out of them 676 were positive (+ve) and 748 were negative (-ve), 388 were males and 288 were females among positive cases. The collected data was coded, computerized and analyzed by SPSS [Statistical Package for Social Sciences] Software version 14.6.

Results

The infection profile based on culture sensitive reports at different health care units such as Government Hospital Laboratory (GHL), Multispeciality Hospital Laboratory (MHL) and a Private pathological Laboratory (PL) have been taken for comparison. The highest numbers of samples were recorded from Government unit (GHL) of 547 which contained about 46.8% of positive to infection, while at Multispeciality hospital laboratory 430 samples were recorded, of them 40.7% were positive to infection. In the Private pathological laboratory about 447 samples were collected and it contained 48.8% samples positive to infection.

The data collected for a period of one year contained six (6) of the common human samples viz urine, stool, blood, sputum, throat swab, pus. In Figure.2, an infection profile in various

pathological samples can be observed. 547 urine samples were recorded which showed 45.5% positive for the presence of infection caused by organisms and 54.5% showed negative which is the absence of organisms. Next to urine sample is 364 pus samples which contained 60.2% positive and 39.8% negative infection. Of 220 blood samples 21.8% showed positive and 78.2% showed negative to infections. The sputum samples of 124 were with 40.3% positive and 59.7% negative infection, and lastly 28 throat swab samples which were recorded with 71.4% positive infection profile and 28.6% negative to infection profile. Out of the 612 infected positive samples, 250 (40.8%) Urine samples, 210 (34.3%) pus samples, 48 (7.8%) sputum samples, 48 (7.8%) stool samples, 37 (6.0%) blood and 19 (3.1%) throat swab samples were recorded at different areas of the Vijayawada city. The above mentioned infected human samples laboratory reports showed the presence of many organisms, out of which five (5) different species were found to be more commonly identified at all the areas. Out of total 45.5% (positive) urine sample, commonest bacterial isolate was E. coli with 49% and least bacterial isolate was P. aeruginosa 4%. The rest were between 10% and 23% as shown in fig.-.2. The positive culture reports showed varied responses to different antibiotics by the organisms in the biological samples. Since urinary tract infections are the most common among the infections, frequent and primary testing of urine sample is advised by the practitioners. So this enrouted for more collection and testing of urine samples for the susceptibility pattern of antibiotics. Thus, the study concentrates on the antibiotic susceptibility of urinary isolates. But to surprise high percentage of resistance is shown towards all most all antibiotics by the organisms. Overall there were about 56 different types of antibiotics used to carry out the culture sensitive tests in different areas. Out of these antibiotics the highest frequency of antibiotics exhibiting resistance and sensitivity together was evaluated (Figure.3). The resistance percentage for the antibiotics was higher than the sensitive percentage except for Amikacin and Azithromycin of 75% and 58.5% respectively. Amoxicillin, Nalidixic acid, Co-trimoxazole, Ciprofloxacin, Norfloxacin, etc are having higher resistance percentage from 70% to 90%. It is a known fact, that Escherichia coli is the commonly isolated organism among the urine samples. The other organisms found to be isolated also were Klebsiella pneumoniae, Staphylococcus aureus, Enterococcus faecalis and Pseudomonas aeruginosa. As per the information of Table.1, the resistance and sensitive patterns of gram negative urinary isolates are observed. Ciprofloxacin, Amoxicillin, Co- trimoxazole, Norfloxacin and Nalidixic acid

were observed to be 100% resistance by P. aeruginosa. While E. coli and K. pneumoniae showed 100% and 96.3% resistance to Amoxicillin respectively. Amikacin and Azithromycin, showed low resistance (between 20.8% and 34.8% respectively) and high sensitivity (79.2% and 65.2% respectively) by E. coli, while K. pneumoniae was susceptible to Amikacin (85.7%) only. The other antibiotics such as Ceftazidime, Kanamycin, Gentamycin, Norfloxacin, etc showed resistance rates from 50% to 85%. Table.2 indicated the resistance and sensitivity of gram positive organisms. S. aureus showed high resistance amoxicillin (72.7%) and showed sensitivity to Amikacin (62.5%) only. E. faecalis showed the resistance rates to be high for Nalidixic acid (88.9%) and Norfloxacin (83.3%). Similar to E. coli, E. faecalis was sensitive to Amikacin (71.4%) and Azithromycin (80%). The rest of the antibiotics such as Kanamycin, Ciprofloxacin, Ceftazidime etc., have shown resistance rate from 50 % to 75% for gram positive organisms.

Discussion
The study was formulated for an outlay of the increasing trends of antibiotic resistance from the clinical sources at specified areas. Various studies on antibiotic resistance are emerging with an intention to bring into lime light about the resistance that is developing among the microorganisms towards the antibiotics. Though the approaches are same, the results are showing entirely different conclusions from time to time. Organisms are showing different responses between old generation and new generation antibiotics, with a change in their genes of both chromosomal and extra chromosomal, which are the causes for them to show resistance to old generation antibiotics and susceptible to newer generation antibiotics. However, reasons for the cause of this resistant activity can be framed out as either more frequent and unnecessary usage of antibiotics or prescribing newer antibiotics with newer combinations for faster recovery of infections. Urinary tract infections UTI is the most frequent clinical manifestation after respiratory tract infections. It has been observed that E. coli is the sole causative agent in more than 80% of uncomplicated UTI [5]. E. coli [6, 7, and 8] and Klebsiella [9] have been reported as the most common organisms causing UTI. In the present study, the top resistance percentage was observed by Amoxycillin (89.6%), Nalidixic acid (86.7%), Co-Trimoxazole (77.3%),Ciprofloxacin (73.4%), Norfloxacin (73.2%). There are studies conducted nationally and internationally regarding the emerging trend in antibiotic resistance. Studies conducted in Eastern Jorden was similar to the present study in organism profile in urine sample and resistance pattern

was high to Co-trimoxazole (59.5%), Gentamycin (43.2%), Nalidixic acid (29.3%) and least to Norfloxacin (11%) and Ciprofloxacin (3.8%) [10]. In another study in Saudi Arabia showed increase in antibiotic resistance between 1999 and 2002 for Ciprofloxacin (between 2% and 35%), Gentamycin (between 2% and 33%), Ceftazidime (between 10% and 15%) by *E. coli*, *K. pneumoniae* and P.aeruginosa [11]. While another study in same Saudi Arabia showed sensitiv patterns for Norfloxacin and Nalidixic acid with similar organism profile to that of the present study [12]. A 15 year reviewed data at Netherlands showed gradual decrease in susceptibility patterns for Co-trimoxazole (from 79% to 62%), Norfloxacin (from 87% to 71%) by *E. coli*, while K.pneumoniae showed 70% resistance to Norfloxacin and 69% resistance to Co-trimoxazole and *P. aeruginosa* was 47% resistance to Norfloxacin.Gentamycin was resistance by Klebsiella and Psedomonas but showed susceptibility by E.coli [13]. *E. coli* showed increased resistance to Norfloxacin and was less resistant to Amoxicillin in an Italian multicentre survey [14]. Another observation in a retrospective study conducted in Iran showed high resistance to Co-trimoxazole by *E. coli* (26.3%) and K.pneumoniae (54.5%), but very least resistance percentage to amikacin (5% to 10%) by *E. coli* and *K. pneumoniae* while gentamycin was less resistant by *E. coli* (5.25%) and highly resistant by K. pneumonia [15]. Studies conducted in India also showed almost similar findings. A study at Kashmir showed more susceptibility to Amikacin and very least susceptibility to Co-trimoxazole by *E. coli* and *K. pneumoniae* [16]. A retrospective study in a tertiary care setting in Bangalore had high resistance to Co-trimoxazole, Amicillin, Amoxicillin and fluroquinolones by *E. coli* [17]. Similar to the present study at Aligarh, Co-trimoxazole topped the resistance pattern (between 40% and 100%), and Amikacin showed the least resistance percentage (between 20% and 51%) by E.coli, *K. pneumoniae*, S.aureus and *P. aeruginosa*. While Ciprofloxacin and Norfloxacin showed resistance by the isolates from 30% to 70% and Gentamycin was resistance from 20% to 70% [18]. In a study in New Delhi topped high resistance patterns of Gentamycin (57.1% to 90%) among the other antibiotics, when compared to the present finding of 40% to 80%. Amikacin showed least resistance when compared to other antibiotics. In contrast to the present study Norfloxacin showed suscpetibility by *P. aeruginosa*, *E. faecalis* and *S. aureus*, while high resistance was observd by gram negative and gram positive organisms [19]. A study in Manipur showed similar finding to the present study an, where *P. aeruginosa* showed 100% resistance to Co-trimoxazole and a contrast finding of sensitivity to

Norfloxacin (72.3%). Another contrast observation was sensitivity to Gentamycin by E.coli [69.5%] and *K. pneumoniae* (76.7%), Sensitivity to Co-trimoxazole (65.5%) and Ciprofloxacin (60.8%) was observed by *K. pneumoniae* [20]. In a study of antibiotic sensitivity and resistance in urinary isolates in Khatmandu valley showed complete contrast observation to the present study where E.coli, *K. pneumoniae* and *S. aureus* were showing sensitive pattern to Amoxicillin (between 64% and 89%), Norfloxacin (between 65% and 75%), Gentamycin (between 90% and 93%) and Cephalosporins (upto 89%). Amikacin was showing susceptibility from (between 80% and 100%) by all the isolates (including *P. aeruginosa*). Report shows that except for Amikacin, P.aeruginosa was resistance to all the antibiotics [21]. Studies have already showed that Nalidixic acid, Norfloxacin and Ciprofloxacin were least effective against all the uropathogens [22, 23]. Further, a study at Nagpur, 82% and 79.6% of urinary isolates to be resistant to co-trimoxazole and ampicillin, respectively indicating maximum resistance to these drugs [24].

Conclusion
The present findings are quite alarming to find almost all antibiotics are showing high percentage of resistance by the urinary isolates. So, this infromation not only relevant for the local area but useful as regional data of refernce for the physicians. Briefly, it can be concluded that based on the highlightings of few observations in a year long survey in Vijayawada on microorganisms which are not responding alike as the same towards antimicrobial agents at all the places. This antibiotic resistance could have developed due to irrational use of antibiotics, and insufficient, irregular use of antibiotics to infections which are not properly diagnised. Further, it is also because of not a proper consumption of new generation antibiotics for the faster recovery from infections. The organisms show resistance to antibiotics that were susceptible to the same antibiotics at the same place of survey. New Drug combination therapy of antibiotics may be followed against organisms. These all facts for the development drug therapy will make awareness in the patients for following laboratory based reports and for prescriptions with the help of doctors. It is also necessary that the patients may always follow the doctors prescriptions rather than their self treatments.

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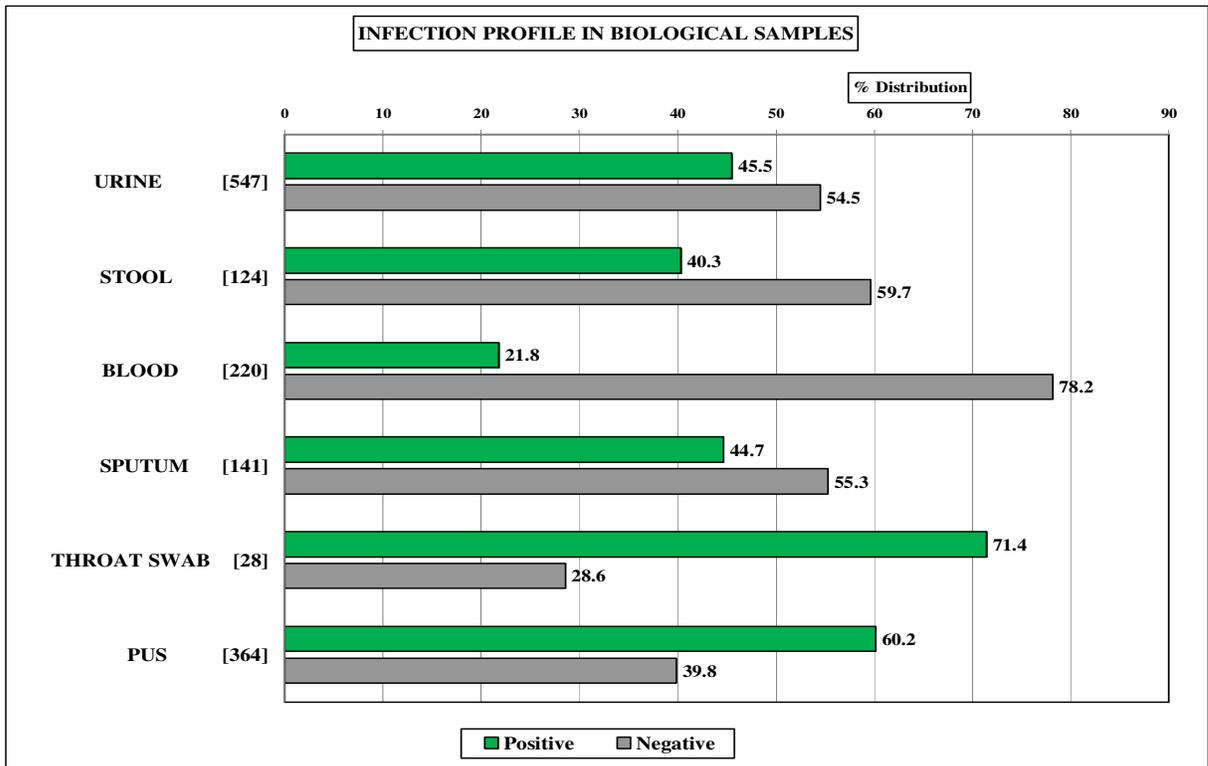[24] Supriya S., Suresh T., Jalgaonkar V., Ahamad S. and Hassani U. (2008) *Indian J Med Res* 120, 553-556.

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INFECTION PROFILE IN BIOLOGICAL SAMPLES

% Distribution

Fig. 1- Percentage distribution of infection profile in 1424 human biological samples collected from a Survey of culture sensitivity reports
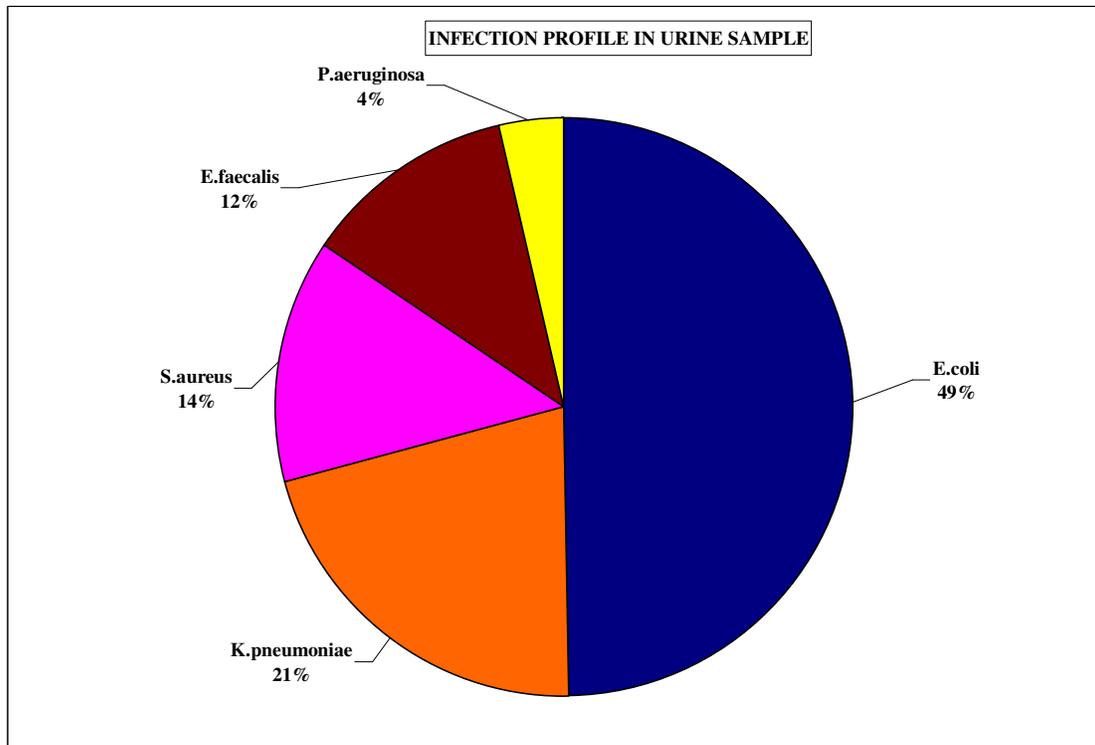
INFECTION PROFILE IN URINE SAMPLE

Fig. 2- Profile of organisms in the positive biological samples collected and Individual organism profile in biological samples collected from the culture sensitivity reports.
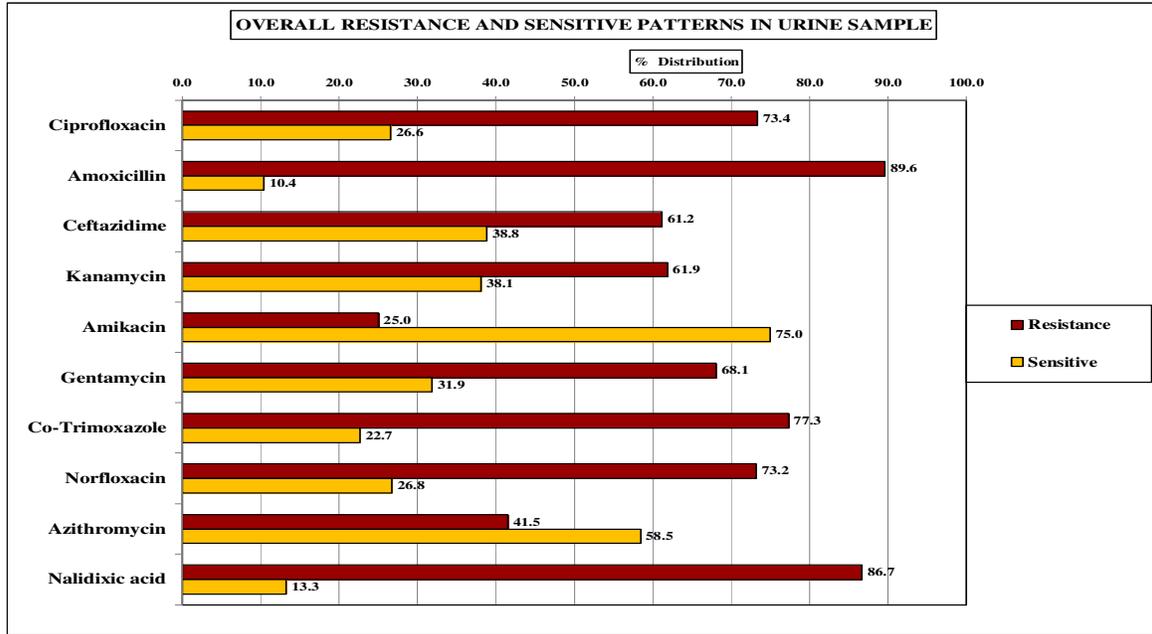
Fig. 3- Antibiotic profile showing overall resistance and sensitive percentage patterns in urine samples.

Table 1- Gram Negative organisms showing resistance and sensitivity to antibiotics in urine sample

Antibiotics	*E. coli*		*K. pneumoniae*		*P. aeruginosa*	
	Resistance %	Sensitivity %	Resistance %	Sensitivity %	Resistance %	Sensitivity %
Ciprofloxacin	91.9	8.1	58.6	41.4	100.0	0.0
Amoxicillin	100.0	0.0	96.3	3.7	100.0	0.0
Ceftazidime	60.0	40.0	57.7	42.3	0.0	0.0
Kanamycin	62.5	37.5	57.1	42.9	80.0	20.0
Amikacin	20.8	79.2	14.3	85.7	50.0	50.0
Gentamycin	61.5	38.5	83.3	16.7	80.0	20.0
Co-Trimoxazole	78.1	21.9	82.4	17.6	100.0	0.0
Norfloxacin	66.7	33.3	82.4	17.6	100.0	0.0
Azithromycin	34.8	65.2	57.1	42.9	66.7	33.3
Nalidixic acid	92.6	7.4	80.0	20.0	100.0	0.0

Table 2- Gram positive organisms showing resistance and sensitivity to antibiotics in urine sample

Antibiotics	*S. aureus*		*E. faecalis*	
	Resistance %	Sensitivity %	Resistance %	Sensitivity %
Ciprofloxacin	63.6	36.4	60.0	40.0
Amoxicillin	72.7	27.3	63.6	36.4
Ceftazidime	60.0	40.0	71.4	28.6
Kanamycin	55.6	44.4	63.6	36.4
Amikacin	60.0	40.0	28.6	71.4
Gentamycin	44.4	55.6	72.7	27.3
Co-Trimoxazole	62.5	37.5	69.2	30.8
Norfloxacin	62.5	37.5	83.3	16.7
Azithromycin	37.5	62.5	20.0	80.0
Nalidixic acid	66.7	33.3	88.9	11.1