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Original Article

Antimicrobial Resistant Pattern of Gram Negative Bacteria to Third Generation Cephalosporins in Rural and Urban Centres of Tamil nadu, India

T. Jeyaseelan Senthinath[1], P. Revathi[2], P.K.Rath[3], M. Suresh[3], R.S.Vigneshwari[4], A.Uma[1], P.T.K.Subramanian[4], K.Moorthy[5*] and N.Thajuddin[6]

[1]Department of Microbiology, Chennai Medical College Hospital & Research Centre (SRM group), Irungalur, Tiruchirapalli - 621 005.
[2]Department of Pharmacology, Chennai Medical College Hospital & Research Centre (SRM group), Irungalur, Tiruchirapalli-621 005.
[3]Department of Microbiology and Molecular Biology, Doctors' Diagnostic Centre, Tiruchirappalli - 620 018.
[4]Department of Medicine, Chennai Medical College Hospital & Research Centre (SRM group), Irungalur, Tiruchirapalli-621 005.
[5]Department of Microbiology, Vivekanandha College of Arts and Sciences for Women, Elayampalayam - 637 205
[6]Department of Microbiology, Bharathidasan University, Tiruchirappalli-620 024.

*Corresponding author: moormicro@gmail.com

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Abstract

Antimicrobial resistance (AMR) increasing now a days and the status in rural areas are less known. The present study aimed to determine the antimicrobial resistant pattern of clinical isolates of selected gram negative bacteria (GNB) to 3rd generation cephalosporin (3rdGCPs) at a rural and urban tertiary care hospital in South India. A total of 710 (Rural -170; Urban-540) isolates (various clinical samples - pus, blood, urine and tracheal fluid) of GNB collected over three consecutive months were studied for AMR pattern to selected. Antibiotic resistant pattern of GNB was very high to3rd GCPs, especially Cephotaxime and Ceftriaxone, less to Ceftazidime among rural isolates campared to urban isolates. As the AMR pattern was high in rural areas, the threat to AMR has caused great concern. This may be due to the irrational prescribing, polypharmacy, dysregulation of marketing systems, self-medication and lack of legislative framework for antibiotic prescription are the key factors for the development of antibiotic resistance. Hence there is an urgent need to design, implement antimicrobial policy and surveillance systems at regional & institutional level under national guidelines. A strategy has been described for antimicrobial stewardship.

Key words: Third Generation Cephalosporin, Gram Negative bacteria, Antimicrobial Resistance, Southern India, Rural and urban community, Antimicrobial stewardship-recommendation.

Introduction

Over the past several decades, the frequency of antimicrobial resistance (AMR) and its association with serious infectious diseases including hospital-acquired Gram-negative infections (Jones, 2001) have increased at an alarming rate (Kim *et al*., 2003). Gram-negative bacteremia is a serious infection associated with significant morbidity and mortality. The worldwide epidemic of antibiotic resistance has affected all patients and felt by medical practitioners. Several studies estimated the burden of antibiotic resistance and reviewed their status (Gould, 2009) and rapid increase in its incidence was reported (Gould, 2007). It is an ecological disaster of unknown consequence and unlike global warming has no obvious solution (Cosgrove *et al*., 2006). The irrational use of antibiotics is well described, but reversing entrenched prescribing practices is extremely difficult (Neu, 1992). The antibiotic pipeline has almost run dry, with no new classes of agent is expected to be in use in the next 20 years (Cosgrove *et al*., 2006). In consequence, antimicrobial resistance to currently available antibiotics is a significant problem world-wide including India. The problem is particularly

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important with the β-lactam antibiotics because of their widespread use (Javier Garau *et al*., 1997). Third generation cephalosporins (3GCPs) were introduced in the Caribbean between the late eighties and the early nineties (Pop-Vicas and D'Agata, 2005) and have emerged as one of the most significant groups of antimicrobial agents available for clinical therapy (Levett *et al*., 1993). The liberal use of 3GCPs in these hospitals has been associated with development of extended-spectrum beta- lactamases (ESBLs) presenting concerns for bacterial resistance in therapeutics. There are limited studies found in southern India on 3GCPs resistance. The purpose of this study is to find out the AMR pattern of selected GNB to 3GCPs with special reference to rural and urban area of Trichirappalli and to identify the variations among rural and urban population.

Materials and Methods

710 strains of GNB (170 - rural and 540 - urban centre) were isolated from various clinical samples such as urine, sputum, pus and other body fluids over a period of three consecutive months (December 2010 to February 2011) from two medical diagnostic centres *viz* . , Department of Microbiology of Chennai Medical College Hospital & Research Centre in Irungalur village and Doctors Diagnostic Centre (DDC), Tiruchirappalli. The former served sampling site for rural community and later for urban. Of these total isolates, 170 belonged to rural and 540 to urban centres *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, were considered for present study. The study ensures that both rural and urban centre followed quality control and good clinical practice (GCP) rules / discipline.

Sterile precautions were advocated (Shanahan *et al*., 1996) to collect samples from the patients with no previous history to identify the status of AMR. The clinical isolates were cultured and identified by standard bio-chemical tests (Chaudhury, 2003). Antibiotic sensitivity was determined by disc diffusion method (Bauer-Kirby *et al*., 1966). Antimicrobial resistance pattern of 3rd generation Cephalosporins (Cephotaxime, Ceftriaxone and Ceftazidime) to the above isolates were analysed. In the present study AMR is defined as bacterial isolates derived from patients and resistant to selected group of antimicrobials. We selected Cephotaxime (CTX), Ceftriaxone (CTR) and Ceftazidime (CAZ) as they are preferred by most of the practicing doctors in the study area. Antimicrobial resistance pattern of these third GCPs belong to rural and urban isolates were compared for each other. AMR variations among rural and urban area of each isolate was determined by Chi-square test and P value also calculated.

Results

The isolated organisms and their resistant pattern to CTX, CTR and CAZ in relation to rural and urban isolates are depicted in table-1 and fig -1-2.

Table- 1: Isolates and AMR: rural and urban variations

Organism	Total Isolates		Isolates in number and AMR pattern (%) to 3GCPs					
			CTX		CTR		CTZ	
	Rural	Urban	Rural	Urban	Rural	Urban	Rural	Urban
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Escherichia coli	120 (70.%6)	310 (57.4%)	104 (88.6%)	130 (41.9%)	108 (90%)	148 (47.7%)	40 (33.3%)	136 (43.9%)
Klebsiella pneumoniae	29 (17%)	135 (25%)	29 (100%)	66 (48.9%)	22 (75.8%)	61 (45.2%)	9 (31%)	40 (29.6%)
Pseudomonas aeruginosa	21 (12.4%)	95 (17.6%)	12 (57.1%)	30 (31.5%)	11 (52.3%)	39 (41%)	4 (19%)	24 (25.3%)
Total	170	540						

Cephotaxime: CTX; Ceftriaxone: CTR; Ceftazidime: CAZ, AMR: Anti-Microbial Resistance: 3GCPs – 3rd Generation Cephalosporins

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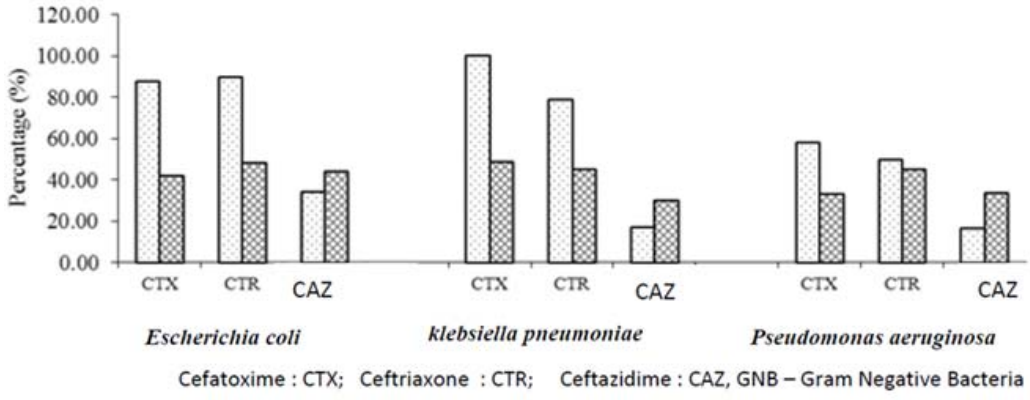

Fig.1: Drug effect on GNB organisms in rural and urban differentiation

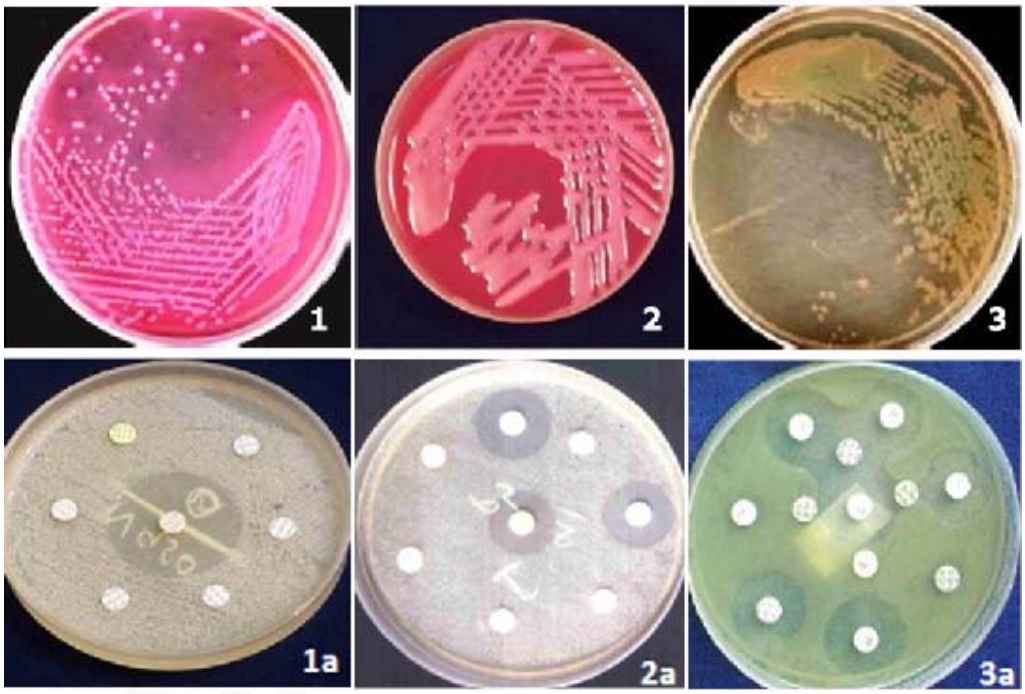

Fig.2: Antibiotic effect of Cephalosporins against GNB organisms

A total of 710 clinical isolates were studied for AMR pattern, among them, 540 (76.05%) were isolated from Urban Centres and 170 (23.94%) were isolated from rural area. Among the three isolates, *Escherichia coli* 430 (60.56%), *Klebsiella pneumoniae* 164 (23.09%) and *Pseudomonas aeruginosa* 116 (16.33%) were isolated from both rural and urban area respectively. Based on antimicrobial resistance pattern, *E.coli* predominates, followed by *K.pneumoniae* and *P.aeruginosa* against Cephotaxime, Ceftriaxone and Ceftazidime. Statistical analysis revealed that *E.coli, K.pneumoniae* and *P.aeruginosa* isolated from rural centres were resistant to CTX significantly ($P > 0.0001$, $P > 0.0001$ and $P > 0.04$ respectively) and for *E. coli, K. pneumoniae* to CTR ($P > 0.0001$ and $P > 0.0037$) more than their urban counter parts. Interestingly the resistance pattern of *E. coli* (43.9%) and *P. aeruginosa* (25.3%) isolates of urban centre were showed high resistant to CAZ in contrast to rural areas but the difference was not statistically significant ($p > 0.7$). There is no statistical difference with

reference to AMR for *P. aeruginosa* to CTR and CTZ, irrespective of the centre from where it was collected.

Discussion

Tertiary care hospitals are a potential source for development and spread of bacterial resistance being in the loop to receive outpatients and referrals from community nursing homes / primary health centres or nearby hospitals (Levett *et al*., 1993). The extensive use of third generation cephalosporin antibiotics has caused the emergence of extended-spectrum-beta-lactamases in Gram-negative bacteria worldwide (Chaudhury, 2003; Koneman *et al*., 1997; Cosgrove *et al*., 2002; Sanders and Sanders, 1992). With regard to GNB, the emergence of extended-spectrum-beta-lactamases producing organisms has resulted in increased morbidity and mortality, as well as increased health care costs. Currently, for the most resistant Gram-negative bacterial infection we have limited or no alternatives in the antimicrobial pipelines for the most resistant GNB infection. AMR needs to be considered a priority by clinicians and administrators alike. Minimizing or eliminating the use of certain antimicrobial agents has been shown to be an effective strategy for dealing with 3GCPs– resistant GNB. Also 3GCPs are being widely used in hospitals for empirical and prophylactic therapy and as their use extends across the broad, more organisms will develop resistance to them presenting the threat of antimicrobial ineffectiveness in life threatening infections (Pfaller *et al*., 1999). β- Lactamase antibiotics (Cephalosporins) are the most varied and widely used agents accounting for 50% of antibiotic usage. β -Lactamase production is the most prevalent mechanism of resistance exhibited by GNB. The plasmid mediated broad-spectrum β-lactamases found in *Escherichia coli* and *Klebsiella pneumoniae* confers resistance to the Cephalosporins. Our study describes the resistant pattern of selected GNB was more for CTX and CTR in rural isolates and for CAZ to urban isolates. The probable reason for increased resistance to CAZ for urban isolates could be attributed to its higher cost than the other two 3GCPs and rarely prescribed in rural areas than urban area. The prescribing pattern may explain for the increased occurrence of resistance to all the three selected pathogens to Ceftazidime in urban areas. These observations clearly indicate that prescribing pattern definitely influences the occurrence of resistance.

In this study the percentage of resistance was high for *Escherichia coli* and *Pseudomonas aeruginosa* than *Klebsiella pneumoniae.* Paradoxical response, which is the reduction of AMR pattern to one with increase in AMR pattern to others were observed. Goossens, 2000 observed a decrease in CAZ resistant *K. pneumoniae* that was accompanied by an increase in cephotaxime - resistant *Acinetobacter baumannii* following an alteration in the antibiotic formulary. On the other hand, a study had been performed in critically ill patients (Pinto Pereira *et al*., 2004), demonstrates one-year restriction of third-generation cephalosporins was accompanied by an improvement in the antibiotic susceptibility of GNB, especially *E. coli* and *K. pneumoniae*.

Antimicrobial resistance is increasing every year and they have a diverse pattern. Among Indian population Chaudhury *et al.* (2003) reported the resistance pattern of *Pseudomonas* to CTX (50%), Taneja *et al.* (2008) observed the resistance pattern of *E.coli, Klebsiella* to CTX (100%) in Chandigarh but Abhilash (2010) in vellore noted the resistant pattern of *E.coli* and *Klebsiella* to CTX 73.5% and CAZ 72.4%. Our report is the first to do comparative analysis of resistance to *E. coli, K. pneumoniae* and *P. aeruginosa* among rural and urban isolates from Trichy, studies from other centres in India and other countries were indicated that overall resistance to rural isolates to selected antimicrobials may differs depends on the different demographic locations.

The probable reason could be

i. Irrational use of antibiotics by general practitioners and other health care workers
ii. Lack of regulatory system or antibiotic policy to govern / monitor antibiotic prescribing pattern
iii. Vigorous incentive based marketing for prescription writing leading to sub therapeutic or inadequate dose

All these factors contribute to increase AMR in rural areas much more than urban area since they are not aware of AMR. Also rural patients tend to go urban hospitals at times or health care and are likely to dissemination of AMR organism from one area to other area. Since the resistant genes are mostly present in the plasmid, they get easily transferred from one bacterium to another through gene transfer mechanism.The AMR is high among rural isolates and it is a threat to

entire rural community. Because of less availability of newer class of antibiotics we have to either go in for combination of β-lactamase inhibitors with 3GCPs or switchover to another class of antimicrobial agents or invent newer antibiotics for GNB resistant microorganisms (Sader *et al*., 2003 ; Watanabe *et al*., 1992).

In general, if a microorganism develops resistance to the second generation of a class of antimicrobial agents it will be resistant to the first generation of that class too. Therefore, antimicrobials that are more potent will be required to tackle such cases and the use of old generation antimicrobials will not be effective. At present, the available evidences suggest that it is extremely that a microorganism could become resistant to a new generation of antimicrobials, yet remain susceptible to old generation antibiotics of the same class (Biedenbach *et al*., 1999). Hence future studies are required to assess the resistance pattern of 4th GCPs, which will guide to reduce AMR/MDR. This study also highlights another strategy called Antibiotic cycling (or antibiotic rotation) (Jazani *et al.,* 2010), an approach that has been used to reduce the occurrence of antibiotic resistance which appears to be of limited value as a sole intervention. Several institutions have implemented antibiotic control strategies aiming judicious antibiotic use, including the restriction of one or more antibiotic classes. However, published studies have shown conflicting or unpredictable results regarding the effect of similar restrictions on the susceptibility patterns of GNB (FDA rules, 2007) showed reduction in the incidence of Cephalosporin-resistant *K. pneumoniae* after a one-year restriction of cephalosporin use but with an unfavourable effect upon imipenem resistance, the consumption of which was increased more than 100%. To tackle this threat, the health education system should create awareness about AMR among the public, all level practitioners, pharmacists and studies integrating microbiology and pharmacology with epidemiology, population genetics, ecology and mathematical biology should be promoted. Also studies that focus on the association of bacterial mechanisms of antibiotic resistance and surveillance system is needed which link antibacterial resistance and antibiotic use. Research is needed to find the best strategies for the optimal use of antibiotics and to estimate the effect of antibiotic exposure on the risk of emerging resistance, as well as on the dynamics of diffusion in the population (Primavera *et al.,* 2004).

Remedial measures-10 steps

As globalization, medical tourism and doctor shopping have become of the order of the day. Various remedial measures (10 steps) to curbs minimize, eliminate the increasing AMR have been suggested under "Antimicrobial stewardship"

i. *Integrated educational programme:* Involving practitioners and prescribers of all categories and explaining the importance of AMR and improving restructuring the prescription practices
ii. *Exploration on containment of bacterial resistance* : Efforts may be made to explore molecular studies on containment of bacterial resistance
iii. *Diffusion dynamics:* Regular studies may be made to collect, consolidate and analysis the bacterial isolates of nearby areas and compare with other areas to find out the diffusion dynamics of bacterial resistance
iv. *Antibiotic cycling:* The knowledge and attitude of the prescribers have to be constantly monitored and modified through CMEs for containment of AMR through antibiotic cycling which has its own limitation
v. *Inter programme education:* As the antimicrobials are used for different purposes in rural areas by veterinarian and agricultural operator and by other traditional medical practitioner, all shall be brought together for training and containment purpose
vi. *Community participation:* As the community is affected by the AMR, efforts may be made to involve the community for understanding antimicrobial use and eliminate misuse or under use
vii. *Infection control programmes*: Infection control programmes should be effectively introduced in all hospitals and prescribers of all cadres both in urban and rural areas be included for this
viii. *Surveillance:* National policy for surveillance of antimicrobial isolates and AMR has to be designed and data has to be shared from peripheral centre through network surveillance for early identification of AMR and initiates containment measures. Risk prone areas / zones may be identified for intensive

surveillance with education in such areas

ix. *Incentive based marketing:* Prescriber has to adhere ethics in prescribing antimicrobials and should not fall a prey to incentive based marketing, which has to be discouraged / curtailed

x. *Policy to regulate AMR:* Regulatory authorities medical, veterinary and agriculture sectors should join together and design a consensus for infection control, prescribing practices, surveillance activities and community participation

Conclusion

Drug resistance development and spread can be slowed (Neu, 1992). Hence the irrational prescribing, polypharmacy, dysregulated marketing of antimicrobials non-involvement of community and self-medication and lack of legislative framework for antibiotic prescription are the key factors for the development of antibiotic resistance. AMR in one area is a slow and constant threat to other communities due to migrating patients / public who carry the AMR organisms from one area or centre to another.

Limitation of Study

The study was limited to single centre.

Most prevalent organisms and commonly used cephalosporin groups were only concerned.

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