MULTIDRUG RESISTANT BACTERIA IN A TERTIARY CARE HOSPITAL.

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ABSTRACT: BACKGROUND: Antibiotic resistance is a global problem in the hospitals as well as in the community. Selection pressure exerted by overuse of antimicrobial agents is the commonest predisposing factor of development of resistance. Problems faced are especially with Methicillin Resistant Staphylococcus aureus (MRSA), Vancomycin Resistant Enterococci (VRE) and Multidrug resistant Gram-negative bacilli (MDR-GNB). **AIMS:** A study was undertaken to find out the prevalence of all bacteria isolated in this hospital from different specimens, which are resistant to first line antibiotics and their antimicrobial susceptibility pattern with higher antibiotics during a six-month period. **MATERIAL AND METHODS:** All isolates from different specimens were processed by standard techniques and identified by standard biochemical tests. Antibiotic susceptibility was performed on Mueller Hinton Agar (MHA) by Kirby-Bauer Disc Diffusion Method (KBDDM), according to CLSI guidelines. Those resistant to first line antibiotics were further tested for higher antibiotics. For Extended Spectrum β -lactamase (ESBL) detection, double disc synergy method was carried out for all Gram-negative bacilli. **RESULTS:** Out of 2987 bacteria grown, 904 (30.3%) were multidrug resistant bacteria. Resistance to first line antibiotics was 83.4% and resistance to all higher antibiotics tested was 16.6%. Sixty percent of Staphylococcus aureus was MRSA and all were sensitive to vancomycin. Prevalence of VRE was 5.3%. Carbapenem resistant Pseudomonas aeruginosa and Acinetobacter species were 19.1% and 9.8% respectively and 10.1% of Klebsiella species was carbapenem resistant. **CONCLUSIONS:** This study highlights the extensive problem of antibiotic resistance encountered in this hospital. Thus, prudent and appropriate uses of antibiotics are required to reduce the emergence of resistance. Each hospital should also have its own antibiotic policy based on the susceptibility pattern of bacteria at a particular time, which should be reviewed as and when required. Rational antibiotic use and effective infection control practices can go a long way in preventing the development of antibiotic resistance.

KEY WORDS: Multidrug resistant bacteria; Tertiary care hospital.

INTRODUCTION: Antibiotic resistance is a global problem in the hospitals as well as in the community and is listed at the top of CDC's list of emerging infectious threats to the public health. Selection pressure exerted by overuse of antimicrobial agents is the commonest predisposing factor of development of resistance, which apart from increasing hospital costs, is

also responsible for prolonged illness and treatment failures in these patients.¹ “Relentless and Dizzying rise of antibiotic resistance” has contributed in a large measure to the persistence of infections as a major cause of morbidity and mortality.² Primary mode of transmission of antimicrobial resistance in a hospital is patient to patient spread via hands and equipments of health care workers.

Problems faced are especially with Methicillin Resistant *Staphylococcus aureus* (MRSA), Vancomycin Resistant Enterococci (VRE) and Multidrug resistant Gram-negative bacilli (MDR-GNB) like *Klebsiella pneumoniae*, *Enterobacter* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter* species.³⁻⁶

Therefore, a study was undertaken to find out the prevalence of all bacteria isolated in this hospital from different specimens, which are resistant to first line antibiotics and their antimicrobial susceptibility pattern with higher antibiotics during a six-month period, i.e. from July to December 2010.

MATERIAL AND METHODS: After approval from Institutional Ethics Committee, all isolates from different specimens were processed by standard techniques and identified by standard biochemical tests.⁷ Antimicrobial susceptibility was performed on Mueller Hinton Agar (MHA) by Kirby-Bauer Disc Diffusion Method (KBDDM), according to CLSI guidelines.⁸ First line antibiotics put up were amikacin (AK), amoxicillin-clavulanic acid (AC), cefuroxime (CU), cefotaxime (CE), ciprofloxacin (CF) and penicillin (P) for Gram-positive cocci and AK, AG, CE, ceftriaxone (CI) and CF for Gram-negative bacilli. Those resistant to first line antibiotics were further tested for higher antibiotics. For all MRSAs, vancomycin (VA), linezolid (LZ) and netilmicin (NT) were further put up. For all enterococci, VA was put up. For all Enterobacteriaceae and *Acinetobacter* species, imipenem (I), NT, cefpirome (Cfp) and cefepime (Cpm) were put, and for *P. aeruginosa* in addition to the above, piperacillin-tazobactam (Pt) was also put up. For Extended Spectrum β -lactamase (ESBL), Double disc synergy method was carried out for all Gram-negative bacilli.⁹ The results were statistically analysed by Proportion method (OpenEPI.com).

RESULTS: Out of total 12,107 samples received during six months, 2987 bacteria were grown (24.7%), of which 904 (30.3%) were multidrug resistant bacteria. Table 1 shows the sample wise distribution of multidrug resistant (MDR) bacteria isolated during the six-month period. Bar Diagram 1 shows the different MDR bacteria (resistant to first line and all the higher antimicrobials tested) isolated during the same period. On statistical analysis by Proportion method, isolation of MDR bacteria was significant from burns swab, blood cultures, stool cultures, anaerobic cultures, urine cultures and endotracheal secretion cultures.

Table 2 shows the predominant Gram-negative bacilli and Gram-positive cocci isolated during the same period. MDR was also significant in *Acinetobacter* species and *Escherichia coli* amongst overall Gram-negative bacilli; and in *Staphylococcus aureus* and *Enterococcus* species amongst overall Gram-positive cocci by Proportion method. Resistance to first line antibiotics was 83.4% and resistance to all higher antibiotics tested was 16.6%. Table 3 shows the distribution of MDR bacteria in different samples.

Sixty percent of *Staphylococcus aureus* was MRSA. Out of 233 MRSA isolated, all were sensitive to vancomycin. Therefore VISA or VRSA was not reported. Of 233, netilmicin sensitivity was seen in 167 MRSAs (71.7%) and 232 were sensitive to linezolid (99.6%). All the MRSAs were resistant to first line antibiotics.

Amongst the 38 enterococci isolated, 36 were sensitive to vancomycin and 2 were resistant. Therefore prevalence of VRE was 5.3%. VRE was isolated from Foley's catheter tips of 2 patients – one was a 13-year male child suffering from Burkitt's lymphoma and the other an adult female having chronic renal failure and pneumonia.

Susceptibility of the common MDR Gram-negative bacilli to higher antibiotics is shown in Bar Diagram 2. Carbapenem resistant *Pseudomonas aeruginosa* and *Acinetobacter* species were 19.1% and 9.8% respectively. Piperacillin-tazobactam sensitivity of *P.aeruginosa* was only 48.1%. While 10.1% of *Klebsiella* species was carbapenem resistant, carbapenem resistance was not seen in *Escherichia coli*, *Enterobacter*, *Citrobacter* and *Proteus* species. One out of 3 *Providencia* species isolated showed carbapenem resistance. Three *Citrobacter* species, 4 *Proteus* species and 3 *Providencia* species isolated were resistant to all the other antibiotics tested.

Only 5 ESBLs were encountered – 3 *Klebsiella pneumoniae* from neonatal blood cultures and 2 *Escherichia coli* – one from adult blood culture and one from stool of an adult male with diarrhea. Out of 5 ESBLs, one was only sensitive to imipenem and other 4 were sensitive to amikacin, but resistant to other antibiotics tested.

DISCUSSION: MRSA appeared in the early 1960s, soon after introduction of penicillinase tolerant penicillins. Clones diversified and nosocomial pathogen spread into the community.³ Methicillin resistance now exceeds 50% in most tertiary care centers.¹⁰ In a study from South India, prevalence of MRSA from clinical samples was 31.1% (250/803) and of all MRSA, MDR was 63.6%.¹¹ In this study, 60% of *S. aureus* was MRSA and almost 50% (233/474) of MRSA isolates were resistant to first line antibiotics. MRSA was significant amongst the total Gram-positive cocci (Table 2). Though VISA is reported from Japan and US, but vancomycin resistance is very low in India. A study from North India has reported 3.3% VISA.¹² However, all the MRSA isolates in the present study were sensitive to vancomycin (100%) and linezolid sensitivity was also good (99.6%). MRSA were most prevalent in pus/wound swabs and in swabs from burn patients – 84.5% (197/233). Nevertheless, hand washing and other standard precautions have to be taken in order to prevent spread of MRSA in hospitals. Screening of health care workers from time to time and decolonization with topical mupirocin in selected cases are must.¹³ Reckless use of this antibiotic may alter the scenario. This coupled with the emergence of Community Acquired MRSA (CA-MRSA) would pose serious clinical problems with global ramifications.¹⁴

MDR *Enterococcus* species was significant amongst the total Gram-positive cocci. Two VRE were isolated in the present study (Table 2). VRE emerged in late 1980s in France and England and subsequently has been isolated all over the world.⁴ They have evolved and diversified in the past two decades. We encountered only 2 VRE in this study, both from Foley's catheter tips. Prolonged hospitalization, severe underlying diseases and/or immunosuppression, stay in intensive care areas, indwelling urinary or central venous catheter, etc. are the risk factors for the development of VRE.⁴ Resistance of enterococci to first line antibiotics was 23.6% (38/161). Only vancomycin sensitivity was 22.4%. Enterococci are always resistant to penicillin and aminoglycosides, leaving us to use no other alternative than vancomycin, overuse of which leads to development of VRE. Karmarkar et al¹⁵ have reported a very high incidence of VRE (53%) of Van B type, while in a previous study in the same institute incidence of VRE was 1%.¹⁶ Thus VRE problem exists, but in a variable manner in hospital environment. Therefore, prudent use of vancomycin and broad spectrum antibiotics should be

done. Hand washing and standard precautions including isolation of colonized or infected patients at one corner of ward or in a separate ward/cabin should be done.¹³

Most tertiary care hospitals are faced with extensive resistance problems in *E. coli* and *Klebsiella* species. Other multiresistant Enterobacteriaceae too establish themselves.^{5,17} *Pseudomonas aeruginosa* was maximum encountered in pus/wound/burn swabs (128/180). *Acinetobacter* species was mostly seen in respiratory specimens (75/218) (Table 2). Isolation of MDR bacteria was significant from burns swab, blood cultures, stool cultures, anaerobic cultures, urine cultures and endotracheal secretion cultures (Table 1).

Escherichia coli and *Acinetobacter* species were significant amongst the total Gram-negative bacilli (Table 2). Amongst Gram-negative bacteria, carbapenem resistance was seen in *Pseudomonas aeruginosa*, *Acinetobacter* species and *Klebsiella* species. Horizontal spread of resistance factors into environmental Gram-negative bacteria has seen the emergence of MDR *Acinetobacter* and *Pseudomonas* species, wherever looked for.^{17,18} Carbapenem resistance of various Gram-negative isolates have been reported from different parts of India.^{5,18,19} In this study, netilmycin sensitivity amongst the common Gram-negative isolates, varied from 8.8% in *Escherichia coli* to 43.9% in *Acinetobacter* species (Bar Diagram 2). Fourth generation cephalosporins also showed high in-vitro resistance against all MDR-GNB (Bar Diagram 2), leaving us to use only imipenem for serious infections, which will lead to more increase in carbapenem resistance in the future.

Increasing resistance of Gram-negative bacilli to 3rd and 4th generation cephalosporins as well as aminoglycosides and quinolones is alarming, which is mainly due to overuse and misuse of these antibiotics.^{6,17} The major risk factors are prolonged stay in ICU, indwelling invasive devices and prior use of cephalosporins and other broad spectrum β -lactam antibiotics. Prudent use of extended spectrum cephalosporins, avoiding prolonged and repetitive use of antibiotics, using antibiotics wisely and treating infection and not contamination are the only means to reduce development of MDR-GNB.¹³ Moreover, vancomycin and linezolid should not be used for prophylaxis and carbapenems should be reserved for serious infections only.

We have reported 26% ESBL amongst Gram-negative bacilli in 2006-07. Strikingly ESBL producing bacteria is on the decline since 2008. Only 5 ESBLs has been reported in second half of 2010 in this study, though there are reports from India of ESBL-producing *E. coli* and *Klebsiella* species.⁹

It should also be kept in mind that antibiotics are used as growth promoters, prophylactics and therapeutic agents in veterinary medicine. This adds to the antibiotic selection pressure which has resulted in breeding of multidrug resistant pathogens in hospitals as well as in the community.²⁰ Over-the-counter availability of antibiotics, primitive infection control in hospitals and weak or deficient sanitation are other factors which lead to acquisition of antibiotic resistance. In large parts of the country technical infrastructure is also lacking.²¹ Failure to implement simple infection control practices such as hand washing and changing of gloves before and after contact with each patient are also common causes of infection spread in hospitals.

Thus, prudent and appropriate use of antibiotics is required to reduce emergence of resistance. Newer antimicrobial agents can be developed, but very few are coming up and most of the newer agents are expensive and unaffordable by common people.¹³ Barrier isolation precautions reduce transmission of resistant bacteria among hospitalized patients and control outbreaks, but this is not applicable in the community.

CONCLUSIONS: This study highlights the extensive problem of antibiotic resistance encountered in India. It also emphasizes the need for systematic programmes to address the problem and evolve an antibiotic conservation practice.

Active surveillance for MRSA, VRE and MDR-GNB should be done frequently and also contact isolation for all colonized patients should be undertaken.

Each hospital should have its own Hospital Infection Control Committee (HICC) with regular hospital rounds and meetings along with education of professionals and all health care workers from time to time.

Each hospital should also have its own antibiotic policy based on the susceptibility pattern of bacteria at a particular time, which should be reviewed from time to time.

Rational antibiotic use and effective infection control practices can go a long way in preventing development of antimicrobial resistance.

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Table 1. Overall Multidrug Resistant Bacteria (MDR) isolated from different samples during July to December 2010

Sample	Total growth	MDR (%)	'P' value*	Significant(S) /Not Significant (NS)
Pus and wound swabs culture	995	291 (29.2)	0.54	NS
Burns swabs culture	209	141 (67.5)	<0.000001	S
Blood culture	538	106 (19.7)	0.000006	S
Stool culture	99	14 (14.1)	0.0005	S
Cerebrospinal fluid culture	38	11 (28.9)	0.86	NS
Anaerobic culture	143	23 (16.1)	0.0002	S
Urine culture	550	115 (20.9)	0.000008	S
Foley's catheter tips culture	90	33 (44.4)	0.19	NS
Sputum culture	125	51 (40.8)	0.012	NS
Endotracheal secretions culture	174	111 (63.8)	<0.000001	S
Pleural fluid culture	26	08 (30.8)	0.95	NS
Total	2987	904 (30.3)	-	-

*By Proportion Method

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Table 2. Predominant MDR Gram-negative bacilli and Gram-positive cocci isolated during July to December 2010

Bacteria	Total growth	MDR	'P' value*	Significant(S) /Not Significant (NS)
<i>Pseudomonas aeruginosa</i>	369	180	0.01	NS
<i>Klebsiella pneumoniae</i>	338	115	0.40	NS
<i>Acinetobacter</i> species	413	218	0.0003	S
<i>Enterobacter</i> species	141	65	0.18	NS
<i>Escherichia coli</i>	389	41	<0.00001	S
<i>Staphylococcus aureus</i>	474	233	<0.00001	S
<i>Enterococcus</i> species	161	38	<0.00001	S

*By Proportion Method

Table 3. Multidrug Resistant Bacteria isolated from different samples during July to December 2010

Organism (Total no.)	Pus & wound swabs	Swabs from burns	Blood cultures	Stool	Urine	Foley's catheter tips	CSF	Anaerobic	Sputum	ET secrns.	Pl. fluid
<i>Pseudomonas aeruginosa</i> (180)	69	59	04	06	11	01	01	03	06	20	00
<i>Klebsiella</i> species (115)	36	00	17	00	26	08	01	03	13	09	02
<i>Acinetobacter</i> species (218)	44	12	43	00	21	08	08	07	21	52	02
<i>Escherichia coli</i> (41)	00	00	02	08	16	09	00	05	00	00	01
<i>Enterobacter</i> species (65)	09	00	10	00	12	03	01	03	05	21	01
<i>Citrobacter</i> species (06)	00	04	00	00	02	00	00	00	00	00	00
<i>Proteus</i> species (04)	00	00	01	00	00	01	00	02	00	00	00
<i>Providencia</i> species (04)	00	02	00	00	00	00	00	00	00	02	00
MRSA (233)	133	64	15	00	05	01	00	00	06	07	02
<i>Enterococci</i> (38)	00	00	14	00	22	02	00	00	00	00	00

CSF: Cerebrospinal fluid; ET secrns.: Endotracheal secretions; Pl. fluid: Pleural fluid.

Bar Diagram 1: Multidrug resistant Gram-negative bacteria isolated during July to December 2010

Bar Diagram 2: Susceptibility of the common MDR Gram-negative bacilli to higher antibiotics