Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/bknrtd62

PATTERN OF ANTIMICROBIAL RESISTANCE IN ICU ALONG WITH CONCERNS IN FUTURE.

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Background : On World Health Day 2011, WHO's statement read "Combat drug resistance: no action today means no cure tomorrow". Eleven years on, antimicrobial resistance remains a global crisis. Drug resistance claims up to 700,000 lives globally each year, and is poised to reach ten million per year by 2050. Experts have classified antimicrobial resistant pathogens into 3 categories:

1. Multi drug resistant organism (MDRO) - non-susceptible to at least 1 agent in 3 antimicrobial categories
2. Extensively drug-resistant organism (XDRO)—non-susceptible to at least 1 agent in all but 2 or fewer antimicrobial categories.
3. Pan-drug-resistant—non-susceptible to all agents in all antimicrobial categories

Infections with such resistant pathogens have limited therapeutic options and are life threatening. MDROs significantly contribute to mortality and morbidity in ICU patients, with increased duration of hospital stay as well as cost of care.
MDROs are identified through in vitro culture and drug susceptibility tests. Common MDROs in healthcare settings include (MRSA, VRE, ESBL producers, CRE etc.). MDR and XDR organisms are common in Indian health care settings, with a large multi-centric study in the country having identified MDR *Staphylococcus aureus*, *Enterococcus sp.*, *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, and Enterobacteriaceae (*E.coli*, *Klebsiella sp.*) as causes of concern.It is important to know the MDRO pattern of a healthcare institute to devise new antibiotic guidelines and adopt better infection control practices so that nosocomial outbreaks may be reduced.

Materials & Methods including: Samples received from the ICU in the Bacteriology laboratory of urban tertiary health care centre in South India for culture and sensitivity testing were screened for MDRO. Urine samples were screened using CHROM agar, while other samples were cultured using Blood agar and Mac Conkey agar. Isolates were identified by conventional biochemical testing. Antibiotic sensitivity patterns were studied using Kirby Bauer disk diffusion method and interpreted as per CLSI guidelines. Data were analysed using Microsoft Excel software.

Result: Our study found a significantly higher prevalence of MDROs and P-XDROs compared to NMDROs ($P = 0.004$). The predominant isolates were E. coli and K. pneumoniae, showing high levels of resistance while showing susceptibility with carbapenems.

Common resistance mechanisms included PMQR genes, ESBLs and mcr genes, carbapenemases. Cephalosporin and carbapenem resistance were widespread, limiting treatment options to higher level antibiotics that usually are not economical for patients.

Acinetobacter species, many times linked to ventilator-associated pneumonia, showed high resistance and persistence. ICU patients with comorbidities were particularly vulnerable.

The results show the growing threat of AMR, limited antibiotic efficacy, and the need for improved infection control, stewardship, and awareness.

Conclusion: The rise of MDROs and P-XDROs, especially in *E. coli* and *K. pneumoniae*, demands the urgent need for stronger antibiotic stewardship, infection control, and public awareness to address the growing threat of antimicrobial resistance.

Bacterial resistance pattern in ICU and future concerns

Introduction:

Antibiotic resistance is one of the most important issues, the current world is facing. With growing rates and severity of resistance, treating infections is becoming more challenging. Since the discovery of first antibiotic Salvarsan which was discovered in 1910, the usage of various antibiotics has been greatly beneficial to humankind. The average lifespan has significantly improved by 23 years since then [1].

Along with discovery of new antibiotics, new resistances were discovered. This issue has been following us for more than 75 years due to various factors like environment, mutations, evolution of bacteria, abuse of antibacterials, and more. The era of antibiotics and its' resistance is subdivided into 5 major eras. Starting with (i) Concerns on Staphylococcus, discovery of Penicillin and its' wide usage (1945 – 1963), (ii) Discovery of Plasmids and terror Superbugs (1963 – 1981), (iii) Awareness on worldwide antibiotic misuse (1981 – 1992), (iv) End of novel antibiotics discovery (1993 – 2013) – The last antibiotic discovered was daptomycin in 1984 and approved in 2003, and finally (v) The threat of rising antibiotic resistances (2013 onwards) [2].

Throughout the history of past century, usage of antibiotics has been a boon and as well a threat if overused. The genes of resistance are not only transmitted vertically but also horizontally by means of plasmids and bacteriophages. The resistance organisms spread as the susceptible variants are wiped out by the antibacterials. The organisms acquire the mechanism of resistances by means of transformation, transduction and conjugation such as (i) Target modification, (ii) Development of efflux mechanisms against drugs, (iii) Reduced drug uptake, (iv) Development of enzymes against drugs [3].

Handling antimicrobial resistance in intensive care units have been constant issue as the patients are on antibiotics almost always. This makes sure the susceptible population is eradicated and only thus resistant organisms remain and keep the infective status of patients persistent. Thus, nosocomial infections almost always remain resistant to common antibiotics and have MDR status. As of 2020, around 35,000 deaths happen in US alone as per CDC data because of such persistent infections in ICUs [4]. ESKAPE is usually a set of MDR organisms that are notorious for persistent untreatable infections in ICUs. These organisms are *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas Aeruginosa and Enterobacter* species [5]. Such organisms are often the cause of sepsis and increased mortality rates in ICU patients [6].

Our study is conducted from a tertiary care hospital in South India. In developing countries like India, the range of over the counter antibiotics is wide and unregulated. Thus, when patients are admitted in wards and ICUs, they are already infected with MDROs. Here we analysed the incidence rate of MDROs in ICU. We believe such studies would help to build comprehensive protocols on usage antibiotics and its' regulation – in both institution wise and national policies.

Methodology:

The retrospective observational study was conducted in The Oxford medical college hospital and research centre between 2022 January and 2024 September. The patients admitted in all kinds of ICUs (Medical ICU, Surgical ICU, Paediatric ICU, Neonatal ICU and ICCU) are included. The samples which had contamination and had only fungal growth, were excluded. Insufficient samples, samples with no growth and commensal growth were excluded as well. If ICU patients are shifted to wards and if samples were collected from wards, those are excluded as well. Patients received from the emergency department were sometimes sent their samples from ER, such samples were excluded as well.

All kinds of samples are included (sputum, wound swab, tissues, ET tube aspirates, pus, blood, urine, stool). Out of 950 samples from 700 patients, 204 eligible samples from 174 eligible patients were collected. Multiple samples from a single patient were usually of different sites, samples, collected at two different dates or during different infectious episodes. Sample collection, processing, inoculation and identification of organisms were carried out as per standard practices. As the study was conducted retrospectively, the outcomes of patients could not be tracked.

Urine samples were screened using CHROM agar, while other samples were cultured using Blood agar and Mac Conkey agar. Isolates were identified by conventional biochemical testing. Antibiotic sensitivity patterns were studied using Kirby Bauer disk diffusion method and interpreted as per CLSI guidelines.

Isolates were tested for 7 classes of antibiotics namely, (i) Beta lactams / Beta lactam + Beta lactamase inhibitor combination, (ii) Cephalosporins, (iii) Fluoroquinolones, (iv) Cotrimoxazole, (v) Carbapenems, (vi) Aminoglycosides, (vii) Macrolides. The sensitivity analyses did not include minimum inhibitory concentration values.

As per CLSI guidelines, an isolate was marked as resistant for a particular category of antimicrobial if at least one agent of the class, is resistant or intermediate against the said bacteria. Only when the isolate is susceptible to all the agents of a particular category, it is labelled as susceptible organism for respective category of antibiotics [7]. Multi Drug Resistance (MDR) was described as non-susceptibility of at least one agent in each of three or more antimicrobial categories. Extensive Drug Resistance (XDR) was defined as non-susceptibility of at least one agent in all except two or less than two antimicrobial categories. Pan Drug Resistance (PDR) is defined as non-susceptibility to all categories of antimicrobials [7]. As resources to determine the possibility of XDROs we have taken the possible XDROs as P-XDR organisms.

The data were collected and statistically analysed in Microsoft Excel ver. 2016. A P-value of less than 0.05 was considered statistically significant.

Results:

After applying above mentioned exclusion criteria, out of 950 samples from 700 patients, 204 samples from 174 patients were found eligible. The susceptibility patterns of each organism corresponding to each antibiotic group has been comprehensively listed in table 1. All organisms showed significant MDR and P-XDR resistance patterns, except Coagulase negative *staphylococcus* and *Enterococci*. These two organisms were generally susceptible to more than 4 types of antibiotics.

Table 1

ISOLATES	R/S/I	BETA LACTAM/ BETA LACTAMASE INHIBITOR COMBO	CEPHALOSPORINS	FLUOROQUINOLONES	COTRIMOX	CARBAPENAMS	AMINOGLYCOSIDES	MACROLIDES
STAPHYLOCOCCUS AUREUS (13)	R	10	2	7	3	1	2	5
	I	0	0	1	1	0	0	1
	S	3	2	2	8	11	0	5
P value	0.032							
KLEBSIELLA PNEUMONIAE (46)	R	43	42	16	25	15	30	0
	I	0	0	1	2	4	2	0
	S	3	4	17	9	25	14	0
P value	0.0021							
ACINETOBACTER SPS (8)	R	8	8	5	3	6	7	0
	I	0	0	0	0	0	0	0
	S	0	0	3	2	2	1	0
P value	0.0001							
PSUDOMONAS AERUGINOSA (20)	R	12	10	5	5	5	15	1
	I	1	1	0	0	0	0	0
	S	8	9	9	3	15	4	1
P value	0.0044							
ENTEROCOCCI (9)	R	8	3	3	0	0	5	5
	I	0	0	0	0	0	0	0
	S	1	0	0	1	1	3	1
P value	0.0055							
KLEBSIELLA OXYTOCA (12)	R	9	11	6	9	8	10	0
	I	0	0	2	1	1	1	0
	S	1	1	1	0	2	1	0
P value	0.0001							
KLEBSIELLA SPS (14)	R	11	6	5	6	3	4	0

	I	0	0	1	0	0	0	0
	S	1	7	0	1	2	2	0
P value	0.0045							
PSEUDOMONAS SPS (7)	R	6	5	1	1	3	6	0
	I	0	0	0	0	0	0	0
	S	1	2	3	1	4	1	0
P value	0.0089							
E. COLI (38)	R	36	37	18	14	9	22	0
	I	0	1	0	0	1	3	0
	S	0	0	6	10	22	13	0
P value	0.0044							
CITROBACTER SPS (7)	R	7	6	2	3	1	4	0
	I	0	0	1	0	3	1	0
	S	0	1	0	2	3	2	0
P value	0.0298							
GNNF (14)	R	11	11	8	5	11	10	0
	I	0	1	1	0	0	2	0
	S	1	2	2	5	3	2	0
P value	0.0001							
PROTEUS SPS (6)	R	5	5	3	2	0	3	0
	I	0	0	0	0	0	0	0
	S	1	1	2	2	5	3	0
P value	0.0136							
COAGULASE NEG. STAPHYLOCOCCUS (4)	R	3	0	1	2	0	0	3
	I	0	0	0	0	0	0	0
	S	1	0	0	0	0	1	0
P value	0.0265							
STAPHYLOCOCCUS HEMOLYTICUS (1)	R	1	1	0	0	0	1	1
	I	0	0	1	1	0	0	1
	S	0	0	0	0	1	0	0
P value	0.0291							
SERRATIA MARCESCENS (1)	R	1	1	0	0	0	0	0
	I	0	0	0	0	0	0	0
	S	0	0	1	1	1	1	0

P value	0.0615							
SALMONELLA SPS (1)	R	1	1	1	1	1	1	0
	I	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0
P value	0							
STREPTOCOCCUS SPS (2)	R	0	0	0	2	0	0	2
	I	0	0	0	0	0	0	0
	S	0	0	0	0	2	0	0
P value	0.3461							

Table 2 & 3 summarises the number and proportion of the bacteria that belongs to each NMDR & MDR/P-XDR category. With the P value of 0.004, the proportion of isolates that belongs to MDR/P-XDR category shows more prevalence than that of NMDR category. Apart from the species mentioned in the tables, we also detected 2 isolates of NMDR *Streptococcus* species, 1 isolate of each P-XDR *Salmonella* species, NMDR *Serratia marcescens* and *Staphylococcus hemolyticus*. As these isolates were rare but statistically insignificant, they are not included in the calculation of significance.

Table 2

Isolate	**NMDR**	**MDR**	**P-XDR**
Acinetobacter	1	1	6
Citrobacter	4	1	2
Coag. Neg. Staph	3	1	
Enterococci	6	3	
E. coli	6	19	14
GNNF	1	4	9
Klebsiella oxytoca	2	2	8
Klebsiella pneumoniae	9	21	16
Klebsiella sps	8	3	3
Proteus sps	2	3	1
Pseudomonas aeruginosa	10	7	3
Pseudomonas sps	2	3	2
Staphylococcus aureus	4	9	

Table 3

Isolate	**% of NMDRO**	**% of MDRO & P-XDRO**
Acinetobacter	25	75
Citrobacter	57	43
Coag. Neg. Staph	75	25
Enterococci	67	33
E. coli	15	85
GNNF	7	93
Klebsiella oxytoca	17	83
Klebsiella pneumoniae	20	80
Klebsiella sps	57	43
Proteus sps	33	67
Pseudomonas aeruginosa	50	50
Pseudomonas sps	29	71
Staphylococcus aureus	18	82
P-value	0.004	

As the study was conducted retrospectively, the data regarding recovery of patients, number of days stayed in ICU/hospital, or diagnosis of patients were unable to be obtained. This also restricted us to probe about the incidence of nosocomial infections in the said population. During the initial period of study, many antibiotics or antibiotic classes were not used in the test. This exclusion seemed irregular regardless of the isolates. Hence, some of the isolates are reported as resistance or susceptible for a particular antibiotic with the available data.

Discussion:

Our study showed significant prevalence of MDROs and P-XDROs (P=0.004) over that of NMDROs. In developing countries, this posses an even bigger threat. *Klebsiella pneumoniae & Escherichia coli* showed a concerning pattern of resistance with maximum MDR and P-XDR isolates. Significant number of them showed susceptibility only to higher level of antibiotics like Carbapenems.

A study reported that gram negative organisms are more prevalent in Indian subcontinent compared to the west where gram positives are more common [8][9]. Our study reflects the same pattern of results by reporting a predominance of E. coli and Klebsiella. Even now these two organisms are of big menace as they evolve faster to acquire a variety of resistance. They acquire resistance mostly through horizontal transmission. Major resistance pathways include: (i) Extended spectrum beta lactamases (ESBLs) produced by them actively cleaves the beta lactams and cephalosporins, (ii) Carbapenemases, (iii) 16S rRNA methylases – resistance to aminoglycosides, (iv) Plasmid mediated quinolone resistant genes (PMQR genes) and (v) mcr genes – Polymyxin resistance [10]. Meanwhile, Klebsiella pneumoniae operates through similar means, it also has efflux pumps, and resistant structural and functional proteins such us outer membrane proteins, gene replication enzymes, protein synthesis complex, transcription enzymes. They also produce biofilm in order to prevent antibiotic entry into the cell [11]. The fact that these resistance mechanisms are horizontally transmissible and the prevalence of relevant organisms shows, there is a serious inadequacy in management and eradication of bacterial infections completely. As this type of transmission doesn't require evolutionary changes, in undertreated infections, even NMDR isolates can eventually acquire multiple resistance through horizontal transmissions.

With rapidly evolving ESBL organisms, the susceptibility of cephalosporins is rapidly decreasing. This rules out cephalosporins as empirical therapy [9]. While the use of carbapenems is encouraged, recent studies, including ours show a wide resistance pattern towards them, especially with E. coli and Klebsiella [9]. These findings are consistent with our study where these two organisms are resistant against both of the groups of antibiotic agents. This leaves us with options such as Ceftazidime/Avibactam combination, Teicoplanin, or Tigecycline.

Acinetobacter is notorious for ventilator associated pneumonia and for living for a considerably longer period on non-living matters. They acquire resistance after being exposed to inadequate amounts of aminoglycosides, carbapenems and cephalosporins. They also lead to poor prognosis of patients [12] Especially in ICU settings, patients are received with pre-existing co morbidities, which makes it harder to get efficient treatment.

AMRs also needs higher antibiotics which are often expensive and even unavailable in some cases. Some critically ill patients might not tolerate higher doses as well.

Though there is a lack of invention of new antibiotics, other measures have been researched to tackle the issue. There are researches underway to tackle this issue with the use of nanoparticles integrated with antibiotics [13]. Immuno-antibiotics are considered as one of the alternatives to conventional antibiotic therapy [14]. Some novel therapies such as Gepotidacin (bacterial topoisomerase inhibitors), Murepavadin (cell membrane synthesis inhibitor) are in clinical trials. In some infections, bacteriophages are engineered and used effectively. Following the discovery of CRISPR-cas, trials are underway to synthesize bacterial sequence specific antimicrobials. Even vaccines against ESKAPE organisms are underway [12]. Yet these are still underway and yet to be available commonly.

Ensuring the patient is not infected by nosocomial infections is one another important aspect of prevention. Often the causative agents of such infections have already acquired many resistance mechanisms and are difficult to treat. As much as treating the resistant infection is crucial, the prevention is equally important. Apart from sanitisation, sterilization of medical equipment, clothing and belongings, even hospital sewage waters are reported to have high amounts of AMRs [15]. Thus, even sewage treatment plants should be regularly audited in order to prevent the spread of AMRs

Though it is a common knowledge among medical fraternity to complete the prescribed antibiotic course, public awareness on the topic is severely lacking. Most of the common public stop using antibiotics once the major symptoms are beginning to wean. Hence, rising awareness and educating the patients while prescribing antibiotics is crucial.

The issue itself is complex with weaning available solutions, requiring multifaceted solutions. Along with newer inventions, rising awareness is important. There needs to be strict protocols for administering antibiotics and even stricter regulation when it comes for animal farm uses. Common public must be educated in the fair use of antibiotics.

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