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Original Article

PROSPECTIVE STUDY ON MULTIDRUG RESISTANT ORGANISMS IN CLINICAL ISOLATES AND CORRELATION WITH EPIDEMIOLOGICAL ISOLATES AT A TERTIARY CARE HOSPITAL IN HYDERABAD

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ABSTRACT

Antimicrobial resistance (AMR) is major global health concern, with multidrug-resistant organisms (MDROs) contributing to increased morbidity, mortality, and healthcare costs. This study aims to determine prevalence of MDROs among clinical isolates and correlate them with organisms recovered from hospital environment in tertiary care hospital, Hyderabad. Prospective study was conducted from January to June 2025 at Osmania General Hospital, Hyderabad. Total of 11,862 clinical samples (blood, urine, sputum, and exudates) and environmental samples (surface swabs, air, and water) were analyzed. Bacterial isolates were identified using standard microbiological methods. Antimicrobial susceptibility testing was performed by Kirby-Bauer disk diffusion following CLSI guidelines. Isolates resistant to three or more antimicrobial classes were classified as MDROs. ESBL, MBL, Amp C, and MRSA were detected using phenotypic confirmatory tests. Of 11,862 clinical samples, 2,352 yielded bacterial growth. Escherichia coli (26.8%) and Klebsiella spp. (24.1%) were predominant isolates. MDROs accounted for 47.6%(n = 1121), with highest resistance observed in E. coli (70.5%) and Klebsiella spp. (57%). ESBL production was highest in E. coli (60.6%), while MBL production was more common in Acinetobacter spp. (14.5%). MRSA was detected in 35.8% of Staphylococcus aureus isolates. Among 224 environmental swabs, 84.3% were sterile; S. aureus and Klebsiella spp. were each isolated in three samples. Air sampling in operation theatres showed 96.8% satisfactory results, water analysis across hospital was satisfactory. High prevalence of MDROs, among E. coli and Klebsiella spp., underscores need for continuous surveillance, stringent infection control practices, and judicious antibiotic use. Environmental monitoring remains vital in preventing hospital-acquired infections.

Keywords: *Multidrug-resistant organisms, ESBL, MBL, MRSA, Hospital environmental surveillance, HAI*

Introduction:

Antimicrobial resistance (AMR) is one of the top global public health and development threats [1]. The emergence and spread of multidrug-resistant organisms (MDROs) is a concern as options for treating patients with these infections are often extremely limited, and MDRO infections are associated with increased lengths of stay, costs, and mortality. AMR burden forecasts study by the Lancet estimated that in 2050, there will be 1·91 million annual deaths attributable to AMR globally and 8·22 million annual deaths associated with AMR. Cumulatively from 2025 to 2050, the study forecasts 39·1 million deaths attributable to AMR and 169 million deaths associated with AMR [2]. The definition most frequently used for Multidrug resistant bacteria is 'resistant to three or more antimicrobial classes' [3,4].Bacteria develop antimicrobial resistance by several mechanisms such as decreased permeability across the cell wall, efflux pumps, by enzymatic inactivation (Ex: Extended spectrum beta lactamases-ESBL, Metallo beta lactamases-MBL) and by modifying the target sites (Ex: Methicillin resistant staphylococcus aureus-MRSA) [5].Reports from hospitals in India suggest that the prevalence of ESBL producing Gram negative bacteria range between 19% and 60%, and that of carbapenem-resistant Gram negative

bacteria between 5.3% and 59%[6,7]. ESBLs are β-lactamases conferring resistance to penicillins, cephalosporins, and aztreonam (but not to cephamycins or carbapenems) by hydrolyzing these antibiotics and are inhibited by β-lactamase inhibitors such as clavulanic acid. AmpC β-lactamases are cephalosporinases that have the ability to hydrolyze and inactivate cephalosporins, cephamycins, aminopenicillins, and monobactams but are not inhibited effectively by clavulanic acid. Carbapenems are used in treatment of ESBL and AmpC producing infections. MBLs can hydrolyze almost all β-lactams, including extended spectrum cephalosporins and carbapenems [5]. Detection of ESBL and MBL producers, along with documentation of their prevalence in patients and the hospital environment, is essential to understand their spread. This, in turn, enables the implementation of appropriate infection control measures, which can reduce patient morbidity and overall hospital expenditure. Keeping this in mind the present study was conducted in a tertiary care hospital in Hyderabad to study the prevalence of MDR organisms from clinical samples received from patients and correlated epidemiology.

Aim of study:

• To isolate and identify the Multidrug resistant bacteria from clinical samples received in the laboratory for bacteriological culture and sensitivity, and to upload the findings to WHONET surveillance system.

• To correlate the MDROs isolated with organisms isolated from hospital environmental samples including surface, air and water samples.

Material and methods:

Total 1784 blood culture samples, 4536 urine culture samples, 1147 sputum culture samples and 4395 exudate (pus, sterile body fluids) samples were received in the bacteriology section of department of microbiology, Osmania General Hospital during January 2025 to June 2025.

For environmental surveillance a total of 224 surface swabs were collected from different selected locations including operation theatres, surgical ICU's, medical ICU's, post OP wards and, orthopedic, burns wards, medical wards. In operation theatres swabs were collected from surfaces of air conditioners, diathiermy, suction apparatus, boyle's apparatus, mayo's trolley, operation theatre table, light and wall. From wards and ICU's surface swabs include patient bed railings, bed side table, monitor, BP apparatus, thermometer and ventilator. Air sampling was carried out by a microbial air sampler machine with a sheep blood agar plate in high- dependency areas like the operation theatre, ICU, and post-operative ward, to assess environmental sterility following fogging. Bacteriological analysis of water was carried out in selected places like overhead tanks, sumps, metro water supply, RO water plant, dialysis unit and kitchen.

The exudate samples include pus, wound swabs, sterile body fluids like pleural fluid, ascitic fluid, cerebrospinal fluid and knee aspirates. Exudates, urine and sputum samples received from patients were first subjected to Gram staining and then were inoculated on blood agar and MacConkey agar plates. The plates were incubated aerobically at 37º C for 18- 24 hrs, sterile body fluids were plated on Chocolate agar along with blood agar and MacConkey agar and incubated for up to 48hrs. Blood culture processing was done by conventional method as per standard guidelines [5,8,9]. Swabs from Environment were processed by inoculating on Blood agar and MacConkey agar plates for aerobic culture and Robertson cooked meat broth for anaerobic growth. Aerobic cultures were reported at 18-24hrs and 48 hrs of incubation. Bacterial growth from clinical and Environmental samples was identified by its cultural characteristics, colony morphology and biochemical reactions. Antimicrobial susceptibility testing was done on Mueller Hinton Agar (MHA) by Kirby Bauer disk diffusion method as per CLSI guidelines [10].

Bacterial isolates that were found to be resistant to at least three or more antibiotic classes were considered MDROs [3,4]. Gram-negative isolates exhibiting resistance to third-generation cephalosporins by disc diffusion were subjected to ESBL screening and confirmatory testing. Phenotypic confirmation of ESBL production was done by using combined disc method employing two disks: Ceftazidime and a combination of Ceftazidime and Clavulanic acid disc if the difference was ≥ 5 mm between the two disks, the isolate was considered as an ESBL producing as shown in fig.1.

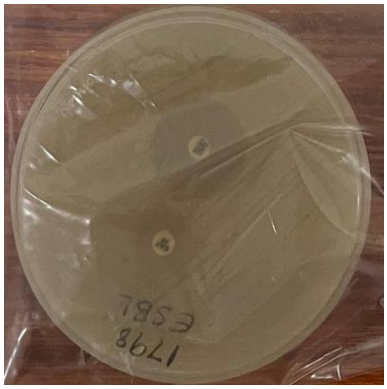

Fig. 1: ESBL detection

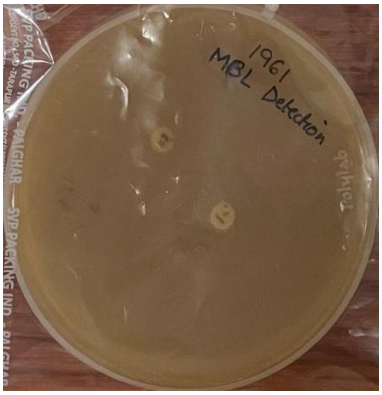

Fig. 2: MBL detection

Fig. 3: Amp C screening

Gram-negative isolates demonstrating resistance to imipenem on initial disc diffusion susceptibility testing were subjected to screening and confirmatory tests for MBL (carbapenemase) production. Phenotypic confirmation of MBL production by combined disc method, Imipenem and a combination of Imipenem and Ethylene diamine tetra acetic acid (EDTA) were employed. If there was a zone of inhibition in the combined disc with Imipenem+ EDTA in comparison to Imipenem alone with a difference of at least ≥7mm between them, the test strain was considered as a MBL producer, fig. 2.

Isolates resistant to cefoxitin (30µg) were screened as AmpC β-lactamase producers, fig. 3.

The patient isolates that which were found to be MDRO were also subjected to colistin testing by agar dilution method.

Fig. 4: MRSA screening

Methicillin Resistant Staphylococcus aureus (MRSA) detection method Cefoxitin disks (5µg) were used as a surrogate for mecA-mediated oxacillin resistance in *Staphylococcus spp.* An inhibition zone diameter of ≤21 mm was reported as methicillin resistant(fig. 4) and ≥22 mm was considered as methicillin susceptible for *Staphylococcus aureus*. A zone of ≤ 24mm was reported as methicillin resistant and a zone of ≥ 25mm was reported as methicillin susceptible *Coagulase Negative Staphylococcus species* (CONS) [10].

Air sampling results were interpreted as per the following criteria:
For the Operation Theatre (OT), environmental sterility was considered satisfactory when bacterial counts were <35 CFU/cubic mm and no fungal colony. For the Intensive Care Unit (ICU) and Post-operative Ward, counts of <50 CFU/cubic mm for bacteria and <5 CFU/cubic mm for fungi were deemed satisfactory.

Bacteriological analysis of water was interpreted as satisfactory when total coliform count was < 3 CFU/100ml and fecal coliform count were nil.

Results:

Of 4395 exudate samples received 1231(30%) showed monomicrobial growth 223(5%) samples showed polymicrobial growth and 198(4.5%) showed contamination. Predominant isolates were Gram negative bacteria, of which 22.6%(279) were *E.coli* 22.1%(273) were *Klebsiella species* followed by 13.7%(169) *Pseudomonas species*, 9.2%(114) *Acinetobacter species* and 4.8%(60) *Citrobacter species.* Among Gram positive isolates *S.aureus* was 17.8%(220) and *Coagulase Negative Staphylococcus species* were 4.6%(57) which were reported only when isolated from implant wound site exudate samples. All the results summarized and depicted below in fig no. 5 & fig.no. 6.

Of 1784 blood culture samples received 221(12.6%) showed growth, 35(2%) showed contamination. *Coagulase Negative Staphylococcus species* 64(29%) were predominantly isolated in paired blood culture samples among Gram positive bacteria. Among Gram negative bacteria *Acinetobacter species* were isolated predominantly 19.5% (43) followed by *Klebsiella species* 15.3%(34), *Pseudomonas species* 12.2%(27), *E.coli* 11.3%(25). Other bacteria included *Enterococcus species* 3.6%(8), *S.aureus* 3.6%(8)

Of 4536 Urine samples received 16.1%(729) showed monomicrobial growth, 8.1%(368) samples showed polymicrobial growth and contaminants. *E.coli* was predominant isolate 44.8%(327) among Gram negative bacteria followed by *Klebsiella species* 22.3%(163), *Pseudomonas species* 5.3%(39) *Citrobacter species* 3.4%(25) , and *Proteus species* 1.4%(10). Among Gram positive bacteria *Enterococcus species* was predominant 19.2%(140) followed by *S.aureus* 2.4%(18) and CoNS 0.2%(2).

Of 1147 sputum samples 15%(171) showed growth , 2.6%(30) showed salivary contamination *Klebsiella pneumoniae* was predominant isolate 57.3%(98) followed by pseudomonas species 21.6%(37) *Acinetobacter species* 10%(17), *S.aureus* 6.4%(11) and *Streptococcus pneumoniae*1.7% (3); as summarized in table no1 below.

Table No.1: Summary of clinical isolates from different samples

	Total samples received	Total positives	S. aureus	CONS	Enterococcus sps	E.coli	Klebsiella sps	Pseudomonas sps	Acinetobacter sps	Citrobacter sps	Others
Exudate	4395	1231	220	57	6	279	273	169	114	60	53
Blood	1784	221	8	64	8	25	34	27	43	9	3
Urine	4536	729	18	2	140	327	163	39	5	25	10
Sputum	1147	171	11	0	0	0	98	37	17	5	3
Total	11862	2352	257	123	154	631	568	272	179	99	69

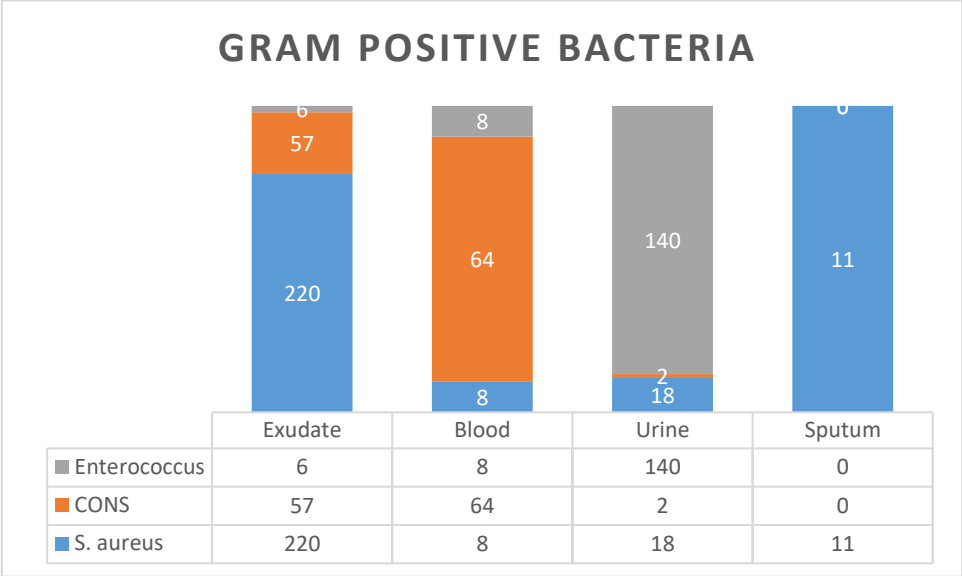

Fig 5: Summary of Gram Positive isolates in total clinical isolates

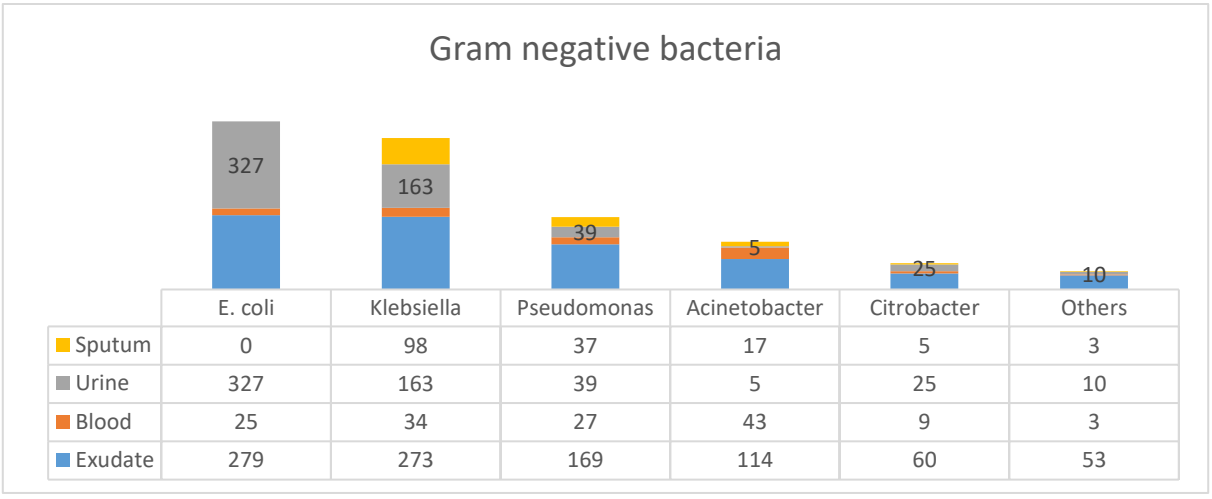

Fig 6: Summary of Gram Negative isolates in total clinical isolates

Table 2: Summary of surface swabs

S.NO	Name of the Ward	No. of Swabs Taken	No. of Swabs Showing Culture sterile	No. of Swabs Showing Culture positive
1	Operation theatres(ortho OT,NS-OT,EYE OT,Septic OT)	86	86	0
2	ICU(RICU,ICCU,AMC,IMC,SICU)	48	12	36
3	Post OP wards (ortho-POW,sugery-POW.NS-POW,ASC-POW	44	11	33
4	Wards(MMW,FMW,FSW,MSW,MOW,FOW)	46	12	34

Table 3: Air sampling results

S.No	Area	Total	Positive	Negative
1	Operation theatre	995	32 (3.2%)	963(96.8%)
2	ICU	123	12 (9.76%)	111(90.2%)
3	Post op ward	218	21(9.6 %)	197(90.4%)

Out of the total 2352 bacterial isolates from various clinical samples, Multidrug resistance was seen in 1121(47.6%) isolates as shown in table no:4 comprising predominantly of *E.coli* 70.5%(n=445) followed by 57%(n=324) *Klebsiella species*, 47%(n=128) *Pseudomonas species* 57.5%(n=103) *Acinetobacter species*, 35.8%(n=92) *S.aureus*. Bacteria including *Citrobacter species*, *Proteus species*, *Enterobacter species*, *Burkholderia species* and *Enterococcus species* also showed multidrug resistance.

Table 4: Summary of MDRO's

Organism	Total Isolated	Total MDRO	ESBL Producers (%)	MBL Producers (%)	MRSA (%)
Klebsiella species	568	57%(n=324)	39.9%(n=227)	7%(n=40)	-
E. coli	631	70.5%(n=445)	60.6%(n=383)	4.5%(n=28)	-
Acinetobacter species	179	57.5%(n=103)	41.3%(n=74)	14.5%(26)	-
Pseudomonas species	272	47%(n=128)	31.6%(n=86)	11.7%(n=32)	-
S.aureus	257	35.8%(n=92)	-	-	35.8%(n=92)

Of the 163 *Klebsiella* species isolated from urine samples 36% (59) were ESBL producers, 8% (13) were ESBL & AmpC producers and 8% (13) were MBL producers. Of 273 *Klebsiella species* isolated from the exudate samples 62.5% (171) were ESBL and 6% (16) were MBL producers. Of 34 *Klebsiella species* isolated from blood samples 25% (7) were ESBL producers.

Out of the 327 *E.coli* isolated from urine samples 60% (197) were ESBL Producers, 12% (39) Both ESBL &Amp C producers and 4.7%(15) were MBL Producers. Of 279 *E.coli* isolated from Exudates 66.7% (186) were ESBL producers. Of 25 *E.coli* isolated from Blood culture samples 33% (8) were ESBL producers.

Of 169 *Pseudomonas species* isolated from exudates 17.6%(30) were MBL producers. Of 27 *Pseudomonas species* isolated from blood 9%(2) were MBL producers.

Of 114 *Acinetobacter species* isolated from exudates, 43 *Acinetobacter species* isolates from blood and 17 *Acinetobacter species* isolates from sputum 12.5%(14), 16.7%(7), 28%(5) were MBL producers respectively.
The MDRO isolates subjected to Colistin agar dilution showed MIC <2µ/dl, which is intermediate according to CLSI fig 7.

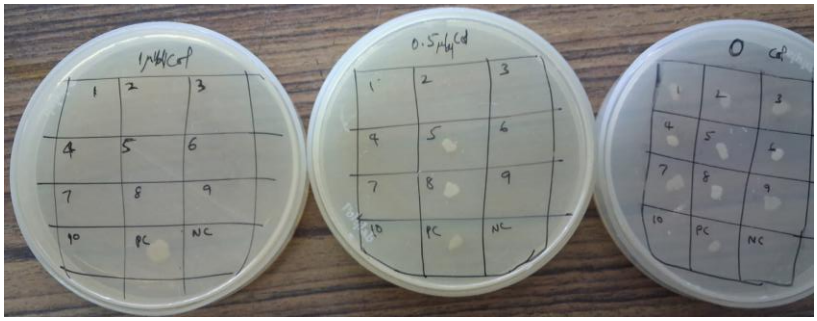

Fig 7 : Colistin agar dilution

Among the environmental surface swabs isolates 189(84.3%) showed no bacterial growth as summerised in table no 2. 20(8.9%) CoNS, 9(4%) Diptheroids, 3(1.3%) S.aureus and 3(1.3%) *Klebsiella species* were isolated from swabs collected from wards. All swabs from operation theatres showed no bacterial growth.

Out of the 3 *S.aureus,* 1 isolate was MRSA. MRSA found on patient-side table. *Klebsiella species* showed sensitivity to all the tested antibiotics. The 3 *Klebsiella species* were isolated from swabs of the same ward indicating potential contamination or hospital associated pathogen.

Among the air sampling results during 6 months of study, operation theatres showed 3.2%(32) unsatisfactory results as shown in table no.3. ICU and post op ward showed 9.76% and 9.6% unsatisfactory results. The areas showing unsatisfactory results were advised to repeat the air sampling after deep cleaning the surfaces, cleaning the air conditioners and fogging the respective areas.
Cleaning and Chlorination of sump was advised where the bacteriological analysis was unsatisfactory.

Discussion:

In the present study among 2352 bacterial isolates 1121(47.6%) isolates were MDRO's. In studies from different parts of the country, prevalence of MDROs ranges from 30% to 60%[7]. Study done by Vishnu Kaniyarakkal et.al showed MDROs at 58% where Predominant MDRO were *E.coli* 66% similar to the present study where MDR *E.coli* are 70.5%[11]. The present study shows 26.8% of *E.coli* from all the clinical samples with 44.8% *E.coli* in Urinary isolates which is in accordance to study conducted and by Kanika Bhargava et al in 2022 (55%)[12]**.** In Exudate samples predominant isolates were Gram negative bacteria *E.coli* 22.6% and *Klebsiella* 22.1% whereas the studies done by Ramya Rengaraj et al in 2021 showed *S.aureus* with 28.4% as the most prevalent pathogen in pus samples[13]. Predominant isolate in paired Blood culture samples was CoNS (29%) similar to the study done by Pradheep Kumar Kokku et al in 2020 with 26.7 % CoNS isolates, Followed by *Acinetobacter species* 19.4%(14). The most common pathogen isolated in sputum sample in the present study was *Klebsiella species* 57.3% followed by *Pseudomonas species* 21.6% and *Acinetobacter species* 9.9% similar to study done by Mahale R P et al in 2024 in which *Klebsiella spp* (47.18%) was the most common pathogen followed by *Pseudomonas* (21.31%) and *Acinetobacter spp*. (17.55%)[15].

In the present study the highest ESBL producing organism was *E.coli* (60.6%) isolated from urine samples similar to the study done by Nagaraj, P. *et al* with 50.4%. ESBL *E.coli* [16].

MBL production was seen in 14.5% of *Acinetobacter species* and 11.8% *Pseudomonas species* isolates similar to the study done by Aradita C, et al with 12.3% MBL production [17] .

In Environmental surface samples 84.3% of swabs were sterile indicating good overall cleanliness. *Coagulase Negative staphylococci* represented 8.9% of isolates. Despite their commensal nature, CoNS have emerged as significant opportunistic pathogens, particularly in nosocomial settings [18]. As this study is conducted at a tertiary care Government hospital where critical and terminally ill patients are referred from other hospitals as a last resort, CoNS in the environment can act as a source of infection in immunocompromised patients. *Klebsiella species* and *S.aureus* were isolated in 1.3% (3) of swabs which are clinically significant. Compared to the study done by G.V. Padmaja et al in 2016 which showed 26% of ESBL's and 10% of MBL's producing Gram negative bacteria in environmental samples [19].The reason in the decline may be attributed to the active infection control committee and regular infection control rounds and surveillance at the hospital. Areas showing unsatisfactory results in Air sampling were given visit by Infection control committee team and advised required corrections.

Conclusion:

There is an increasing emergence of nosocomial infections caused by Multidrug-resistant pathogens, including ESBL and MBL-producing bacteria, which significantly contribute to antimicrobial resistance. This information which is uploaded to WHONET is useful for guiding clinicians in selecting appropriate empirical antibiotic therapy by generating ward specific antibiograms. Conducting regular surveillance studies in all hospitals is crucial for gathering data on the susceptibility patterns of both clinical and environmental isolates. The present study has taken a complete approach by conducting air, water and surface surveillance and correlating these findings with clinical isolates. Studies incorporating both environmental and clinical isolates provide insights into the transmission dynamics of hospital acquired infections and emphasize the necessity of stringent cleaning protocols and preventive measures.

STATISTICAL METHODS:

Descriptive statistics like mean and percentage were used to infer data. Microsoft office 2019 was used to make tables.

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Conflict of interest: the authors declare that there is no conflict of interest.
Author's contribution:
Dr. G.V.Padmaja conceptualized and supervised the study.

Dr. Uzma and Dr. Nazia performed tests, data validation and data curation.
All authors contributed equally writing and approving the final manuscript for publication.
Funding: None
Data availability:
Included in manuscript
Ethics statement: Institutional Ethical clearance taken for the study.

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