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Research Article

Priority Pathogens and its Antibiotic Resistance in various clinical samples at Tertiary Care Hospital

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ABSTRACT

Introduction:
Antimicrobial resistance (AMR) is an urgent public health threat, growing its incidence worldwide by a compression of selection pressure when pathogens exposes to antibiotics and transmission of resistance by various mechanisms. Appropriate identification of these pathogens will help the clinicians for proper diagnosis and treatment. Therefore, we tried to investigate the priority pathogens causing various infections in our tertiary care hospital in India.

Materials And Methods:
A Prospective, cohort study was conducted on 15153 clinical samples during the study period to isolate the priority pathogens at the department of Microbiology. All the samples were collected under aseptic precautions and processed as per the standard protocols. Clinical and Laboratory Standards Institute (CLSI) guidelines were followed during testing time along with quality controls. All the data including microbiological, infection and demographic characteristics were collected in a pre-structure excel sheet.

Results:
Escherichia coli was the predominant organism isolated (30.9%) followed by Klebsiella pneumoniae (25.9%), Klebsiella oxytoca (18%), Pseudomonas aeruginosa (11.3%), MSSA (6.6%), MRSA (4.4%), E.faecium (1.8%), and Acinetobacter baumanii (0.5%). Total of 412 (64.4%) Enterobacteriaceae out of 639 were Extended-Spectrum Beta-lactamases (ESBL) producers, 71.6% of Enterobacteriaceae were resistant to 3rd generation cephalosporins, and 40% of S.aureus isolates were Methicillin-resistant. Carbapenem resistant Pseudomonas aeruginosa were 11.3%.

Conclusion:
These findings can guide to maintain the data at laboratory, priority pathogens its antibiotic resistance to contribute towards local and global understanding of the AMR public health threat and provide guidance for priorities, policy, and practices for the optimal use of antimicrobials and prevent morbidity and mortality of patients.

Keywords*: Antimicrobial resistance, priority pathogens list..*

INTRODUCTION

Antimicrobial resistance (AMR) is an urgent public health threat, growing its incidence worldwide by a compression of selection pressure when pathogens exposes to antibiotics and transmission of resistance by various mechanisms. Survival and spread of these AMR pathogens in human population will result in increase in morbidity and mortality. People are being affected on one side by no effective treatment options to kill these AMR pathogens [1] and another side experience of serious side effects such as organ failure, financial loss and prolonged health care due to usage of second and third-line antimicrobials [2].

AMR can affect people at any stage of life. They can make all antimicrobial ineffective, resulting in unstoppable infections. The most crucial step to save human lives is to stop the spread of AMR.

In 2015, WHO introduced the Global Action Plan on Antimicrobial Resistance and in 2017 the first bacterial priority pathogens list (BPPL) was launched by WHO [3]. This list was created based on resistant profile of pathogens, public health impact, and the need for new treatments. This will help in research and development of new antimicrobials.
The 2024 WHO BPPL covers 24 pathogens, spanning 15 families of antibiotic-resistant bacteria pathogens. Notable among these are Gram-negative bacteria resistant to last-resort antibiotics, drug-resistant *mycobacterium tuberculosis*, and other high-burden resistant pathogens such as *Salmonella*, *Shigella*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* [3].

Indian population is known to be the highest consumer of antibiotics in the world [4]. The AMR situation in India has raised grave public health concerns [5] and an action plan for its control is considered crucial [6,7]. The infectious diseases presentation and its microbiotia can differ significantly across countries, regions with in the country and communities. Appropriate identification of these pathogens will help the clinicians for proper diagnosis and treatment. Therefore, we tried to investigate the priority pathogens causing various infections in our tertiary care hospital in India.

Aim & Objectives:
1.To study the prevalence of priority pathogens among various clinical samples
2.To know the ESBL resistance pattern of *Enterobacteriaceae*
3.To know the MRSA percentage among *S.aureus* isolates.

MATERIALS AND METHODS:

Study Design & Settings:
This is a prospective, cohort study conducted on patients with suspicious of bacterial infections, in and around Guntur, Andhra Pradesh. The study has been carried out in department of Microbiology of Guntur Medical College, Guntur, Andhra Pradesh. An informed consent was taken from the study population prior to the study. All the microbiological data was entered into the microbiology culture registers on regular basis, this data was used for the present study. A total of 15153 clinical specimens from patients suspicious of infectious diseases were processed. Among those isolates of priority pathogens were analyzed further.

Study period: 8 months (January 2024 to August 2024).

Sample Processing:
All the clinical specimens received at sample collection area were immediately transported to laboratory and processed. If there is any delay in processing those samples can be stored at refrigerator for 24 hours. Samples were inoculated onto Nutrient agar, Blood agar, MacConkey agar and Chocolate agar by using standard techniques. Plates were incubated at 37ºC for overnight before the plates were inspected for growth. Gram's staining was performed [8].

Identification of pathogen:
Identification of all isolates was done on the basis of routine biochemical tests i.e., Gram staining, catalase test, oxidase test, motility, indole production, methyl red test, voges proskauer test, citrate utilization test, nitrate reduction test, triple sugar iron test, urease production, sugar fermentation test and amino acid decarboxylation tests using standard techniques.

Antibiotic susceptibility testing:
This was performed using Kirby Bauer disk diffusion method. Following antibiotic disks were used: Amikacin (30 µg), Gentamicin (10 µg), Amoxicillin/Clavulanate (20/10 µg), Ceftazidime (30 µg), Cefepime (30 µg), Cefuroxime (30 µg), Ciprofloxacin (5 µg), Cotrimoxazole (1.25/23.75 µg), Nalidixic acid (30 µg), Nitrofurantoin (300 µg), Norfloxacin (10 µg), Piperacillin/ Tazobactum (100/10 µg), Imipenem(10 µg). The disk was obtained from high media laboratories. The diameter of zone of inhibition was measured and interpreted according to CLSI guidelines (2024) [9].

ESBL detection:
Screening test - ESBL detection was done for all isolates according to latest CLSI criteria. Screening test According to latest CLSI guidelines, zone diameter of Escherichia coli strain for ceftazidime<21mm is presumptively taken to indicate ESBL production.

Confirmatory test - As per CLSI guidelines, ESBLs were confirmed by placing disk of cefotaxime and ceftazidime at a distance of 20mm from a disk of cefotaxime/clavulanate (30/10µg) and ceftazidime/clavulanate (30/10µg) respectively on a lawn culture of test strain (0.5 McFarland inoculum size) on Mueller-Hinton agar. After overnight incubation at 37° C, ESBL production was confirmed if there was ≥ 5mm increase in zone diameter for either antimicrobial agent tested in

combination with clavulanate versus its zone when tested alone [9]. For quality control of antimicrobial susceptibility for disc diffusion *Esch.coli* ATCC 25922 strain was used.

MRSA detection:
MRSA isolates were identified by cefoxitin (30µg) disc testing (HiMedia, Mumbai). Cefoxitin Disc diffusion test was performed by making inoculums of test isolate. Subsequently test isolate was incubated for 2-3 hours and then isolate turbidity was matched to 0.5 McFarland standard. After standardization of inoculum, MHA plate was inoculated for lawn culture and cefoxitin disc 30 µg was placed and plate was incubated for 35°C for 18-24 hour. The < 22mm cefoxitin disk (30µg) zone was considered as Methicillin resistance as per CLSI 2024 guidelines [9]. For quality control of antimicrobial susceptibility for disc diffusion *S. aureus* ATCC 25923 strain was used.

Data collection: Data pertaining to type of infection, clinical manifestations, and microbiological findings including staining features, culture and sensitivity was collected along with general demographic data.

Statistical analysis: The data was entered into Microsoft excel sheet and calculated the results. The statistical analysis such as mean ± standard deviation, frequency, and percentages done in Microsoft excel.

RESULTS:
A total of 15153 clinical specimens from patients suspicious of infectious diseases were processed. Out of 15153 clinical specimens 2609 (17.2%) were culture positive. A total of 852 (32.6%) were identified as a priority pathogens as per WHO, among all culture positive samples (2609) which were considered and reported as pathogens.

Majority of the isolates were isolated from urinary tract infections i.e., 513 (60.2%) out of 852 followed by blood stream infections which were 216 (25.3%) out of 852, 12.08% pyogenic infections and 2.4% infections in sterile sites. *Escherichia coli* was the predominant organism isolated (30.9%) followed by *Klebsiella pneumoniae* (25.9%), *Klebsiella oxytoca* (18%), *Pseudomonas aeruginosa* (11.3%), MSSA (6.6%), MRSA (4.4%), *E.faecium* (1.8%), and *Acinetobacter baumanii* (0.5%) (Table 1).

Escherichia coli (43.8%) were predominantly noted in Urinary tract infections. Among blood stream infections *Klebsiella pneumoniae* (46.2%) was predominant pathogen. *Escherichia coli* (25.2%), *Klebsiella pneumoniae* (19.4%) and *Pseudomonas aeruginosa* (20.3%) were majority pathogens in pyogenic infections.

Table 1. Distribution of WHO priority pathogens isolated in the study population

Organisms	Blood (n=216)	Urine (n=513)	Sterile body fluids (n=21)	Pus (n=103)	Total
Escherichia coli	12	225	1	26	264 (30.9%)
Klebsiella pneumoniae	100	91	10	20	221 (25.9%)
Klebsiella oxytoca	17	128	1	8	154 (18%)
Acinetobacter baumanii	2	1	0	2	5 (0.5%)
Pseudomonas aeruginosa	15	55	6	21	97 (11.3%)
MRSA	27	0	1	10	38 (4.4%)
MSSA	40	0	1	16	57 (6.6%)
E.faecium	3	13	0	0	16 (1.8%)

Total of 412 (64.4%) *Enterobacteriaceae* out of 639 were Extended-Spectrum Beta-lactamases (ESBL) producers, 71.6% (458 out of 639) of *Enterobacteriaceae* were resistant to 3rd generation cephalosporins, and 40% (38 out of 95) of *S.aureus* isolates were Methicillin-resistant. Carbapenem resistant *Pseudomonas aeruginosa* were 11 (11.3%) out of 97 (Table 2).

Table 2. Resistant patterns of Priority pathogens isolated in this study

Drugs	Organisms resistant to	% of resistance
ESBL	*Enterobacteriaceae*	64.4%
3rd Generation cephalosporins	*Enterobacteriaceae*	71.6%
Methicillin	*Staphylococcus aureus*	40%
Carbapenem resistant	*Pseudomonas aeruginosa*	11.3%

DISCUSSION:
The prioritization process involved multi-criteria decision analysis (MCDA) which used information from multiple sources, including disease mortality, transmissibility, treatability, health care burden, preventability in health care

settings, and preventability in community settings, etc. Twelve families of drug-resistant bacteria, posing the greatest threat to human health, were categorized as critical, high, and medium priority organisms, in terms of their resistance to selected antimicrobials [10].

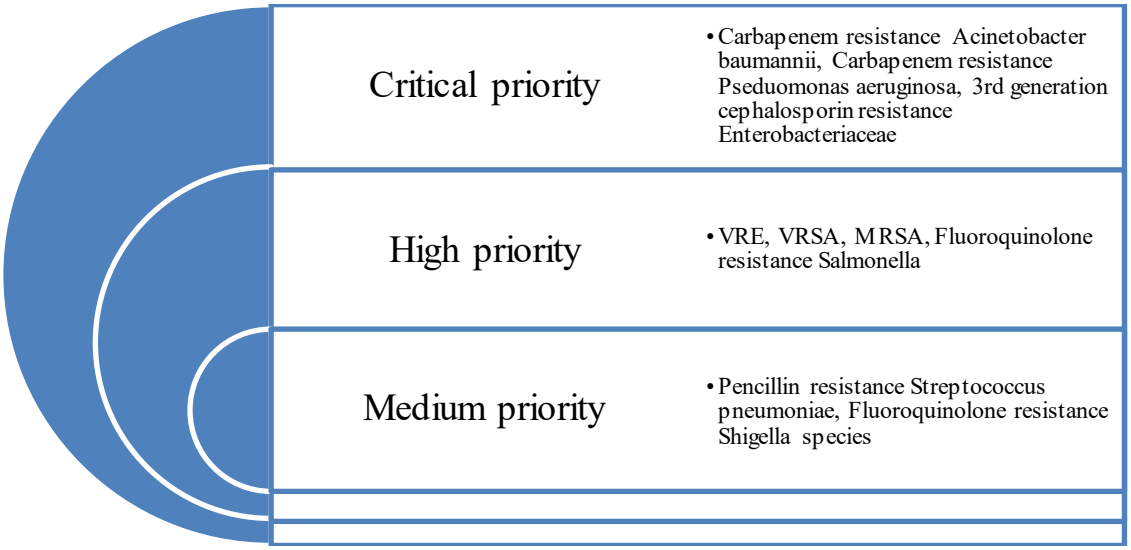

Fig 1. Priority pathogens list as per WHO

Out of 15153 clinical specimens 2609 (17.2%) were culture positive. A total of 852 (32.6%) were identified as a priority pathogens as per WHO among culture positive specimens. Mogasale VV et al [11] did study on WHO priority pathogens which were isolated in outpatient and inpatient department children, they studied a total 12,256 samples which showed 19% culture positive and 1556 (66.6%) were priority pathogens out of 2335 culture positive specimens.

Majority of the isolates were isolated from urinary tract infections 60.2% followed by blood stream infections 25.3%, 12.08% pyogenic infections and 2.4% infections in sterile sites. *Escherichia coli* was the predominant organism isolated (30.9%) followed by *Klebsiella pneumoniae* (25.9%), *Klebsiella oxytoca* (18%), *Pseudomonas aeruginosa* (11.3%), *MSSA* (6.6%), *MRSA* (4.4%), *E.faecium* (1.8%), and *Acinetobacter baumanii* (0.5%). A study from South India [11] revealed as *E. coli* was the most common organism isolated (576), followed by *Staphylococcus aureus* (252).

In this study a total of 64.4% *Enterobacteriaceae* were Extended-Spectrum Beta-lactamases (ESBL) producers, 71.6% of *Enterobacteriaceae* were resistant to 3rd generation cephalosporins, and 40% of *S.aureus* isolates were Methicillin-resistant. Carbapenem resistant *Pseudomonas aeruginosa* were 11 (11.3%) out of 97. Mogasale VV et al [11] noted among the main WHO PPL organisms identified, 72% of *E. coli* and 63% of *Klebsiella* spp. were resistant to 3rd generation cephalosporins due to extended-spectrum beta-lactamase (ESBL), and 53% of the *Staph. aureus* were Methicillin-resistant. Overall, nearly half of *Enterobacteriaceae* were resistant to carbapenem (46%) or 3rd generation cephalosporins due to ESBL (55%). The carbapenem resistance in *Pseudomonas aeruginosa* was found low (5%).

Research workers on their toes on isolating the drug resistant pathogens and assessing its antimicrobial susceptibility to help in the management of infectious diseases cases and framing antimicrobial stewardship programme. WHO classified *Acinetobacter baumannii* and *Pseudomonas aeruginosa* as critical priority pathogens in 2017 after notifying these pathogens global prevalence as 91% and 82% respectively [11]. ESBL producing *Pseudomonas aeruginosa* were 42.3% and 22.2% by Goel Varun et al [12] and Agarwal et al [13] and the MBL production was 24% and 28% by Nagaveni et al [14] and Anuradha et al [15].

41.4%. Kaljita JM et al [16] noted 74.79% of *Klebsiella* and 74.32% of *Acinetobacter species* were MDR bugs. *MRSA* was observed in 13.26% isolates and Inducible clindamycin resistance among *Staphylococcus aureus* isolates was observed in 16.19%. Upreti N et al [17] stated among *S. aureus* isolates, 60.6% were *MRSA* strains, whereas 40% of *K. pneumoniae* and 33.3% of *C. freundii* were ESBL producing bacteria followed by *E. coli* (25%). Ogefere et al. [18] have also reported a high resistance against amoxicillin-clavulanate, ceftazidime, ceftriaxone, gentamicin, ciprofloxacin, and ofloxacin by ESBL producers. A study from Europe showed considerable variation, ranging from 1.6% (Latvia) to 23.2% (Russia), in the prevalence of ESBL-producing *E. coli* isolates [19].

Resistant pathogens can affect the patient's quality of life and their financial status, they also disturbs environmental resources. The strength of this study is to find the prevalence of priority pathogens in this community which will aid to reduce the spread of infection and misuse of antibiotics. Further more research works on the prevalence of priority pathogens in various regions, its predominant habitat and the spread of infection ways will give a clear cut suggestions on

how can we prevent these pathogens. Therefore, state or central government health authorities must consider all the factors responsible for spreading of pathogens in the India which will help to initiate stringent measures on infection control practices and antimicrobial stewardship activities.

CONCLUSION:

Critical priority pathogens including Extended-Spectrum Beta-lactamases (ESBL) producers of *Enterobacteriaceae*, third generation cephalosporins resistant *Enterobacteriaceae*, Carbapenem resistant *Pseudomonas aeruginosa* and the high priority pathogen Methicillin-resistant *Staphylococcus aureus* were observed in various clinical samples.

These findings can guide to maintain the data at laboratory, priority pathogens its antibiotic resistance to contribute towards local and global understanding of the AMR public health threat and provide guidance for priorities, policy, and practices for the optimal use of antimicrobials and prevent morbidity and mortality of patients.

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