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A DESCRIPTIVE STUDY OF ANTIMICROBIAL RESISTANCE PATTERNS OF COMMON PATHOGENS ISOLATED IN TRIHMS, TERTIARY CARE HOSPITAL IN ARUNACHAL PRADESH.

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ABSTRACT The widespread uses of antibiotics, together with length of time over which they have been available, have led to emergence of resistant bacterial pathogens contributing to morbidity & mortality. This study aimed to assess bacterial isolates and their antimicrobial susceptibility patterns in a tertiary care centre of north-east India. **Methodology:** A cross sectional hospital based study was done. Samples collected in lab were processed for identification of bacteria and antimicrobial susceptibility testing by Kirby-bauer disk diffusion method. **Result:** A total of 1609 samples were processed. Majority of samples were urine(64.2%) and pus(27.65%).Staph aureus(48.72%), *Escherichia coli* (25.95%), *Enterococcus spp.* (7.63%), *Klebsiella spp.* (6.64%), *Citrobacter spp.* (3.74%), *Pseudomonas spp.* (3.46%),were commonly isolated pathogens. Most isolates were resistant to β-lactam antibiotics & cephalosporin group of drugs. **Conclusion:** In this study, Staph aureus & E coli were commonly isolated pathogens with high resistance towards β-lactam antibiotics & cephalosporins.

KEYWORDS : Antimicrobial Resistance (AMR), Global Burden Of Death (GBD), Urinary Tract Infection (UTI), Blood Stream Infection (BSI), Pathogens, Antimicrobials, Species (Spp).

INTRODUCTION:

Antimicrobial resistance (AMR) poses a major threat to human health around the world with highest burdens in low-resource settings.[1] Bacterial antimicrobial resistance (AMR)— occur when changes in bacteria cause the drugs used to treat infections to become less effective. Excessive prescription by physician without indications, self-medication in community and intensive and prolonged use of antimicrobials in hospital setting estimates the effect of AMR on incidence & prevalance.[2] WHO outlines 40 research topics on drug-resistant bacteria, fungi and *Mycobacterium tuberculosis* to be answered by 2030, in line with Sustainable Development Goals (SDG). AMR related mortality was 1.27 million in 2019 globally. It affects all regions and all income levels.[3]

In 2019, India had 145th highest age-standardized mortality rate per 100,000 population associated with AMR across 204 countries. In Global Burden of Death region of South Asia, India has 3rd lowest age-standardized mortality across 5 countries.[4] There is an increasing trend of development of resistance to commonly used antimicrobials in pathogens like *Staphylococcus aureus, Enterococcus spp, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeroginosa, Salmonella, Shigella, N. gonorrhoeae, N. meningitides, Mycobacterium tuberculosis*. Resistance spectrum of pathogens varies with population, time, geographical location and hospital ward. Various studies in South India highlighted drug resistant pattern like multidrug resistant Extended-Spectrum β-Lactamase Producing *Klebsiella pneumoniae, vancomycin-intermediate staphylococci*, fluoroquinolone resistant *Pseudomonas aeruginosa, etc.*[5]

Staph aureus is a frequent coloniser of human population and one of foremost opportunistic bacterial pathogens of human. Multidrug resistant staph auresus is a massive concern in clinical world. Enterococcus spp have gradually evolved from commensals to cause life-threatening hospital acquired infection. There is not much data on high incidence of vancomycin resistant *Enterococci* (VRE) in India. *E. coli* is intrinsically susceptible to almost all clinically relevant antimicrobial agents, but this bacterial species has a great capacity to accumulate resistant genes, mostly through horizontal gene transfer.[6] WHO listed Klebsiella pneumoniae as one of the AMR bacteria of concern due to its demonstration proclivity for developing antimicrobial resistance to many classes of antibiotics such as penicillin, cephalosporin, quinolones.[7] Antimicrobial resistance in pseudomonas aeruginosa is usually the result of combination of different imported (mobile genetic elements) and chromosomally encoded resistant mechanism.

Datas on study of antimicrobial resistance in different part of north-east are not readily available. In our study we highlighted antimicrobial susceptibility pattern of five commonly isolated pathogens of global concern.

AIM:

To evaluate the antimicrobial susceptibility patterns of commonly isolated pathogens in Tomo Riba Institute of Health & Medical Sciences (TRIHMS).

OBJECTIVES:

- To find out the common pathogens isolated in TRIHMS.
- To find out the most susceptible & resistant group of antimicrobials of that pathogens.

MATERIALS & METHODS:

Here we worked on pathogens isolated from various samples. Specimens were collected from patients of different departments of hospital in bacteriology lab. These were urine, blood, pus & other body fluids (like CSF, ascitic fluid, pleural fluid, synovial fluid, sputum, broncho-alveolar lavage, bronchial brashing, gastric lavage, swabs), sputum and stool.

Bacterial Identification:

Bacterial strains were identified by conventional microbiological methods. Here we prepared a smear from specimen received and did a gram stain, then inoculated on nutrient agar(NA), MacConkey agar(MA), Blood agar(BA) in case of blood, pus or other body fluids& CLED if the sample was urine, then incubate at 37°C for 16-18 hours. We prepared smear from pure isolated colonies and did gram stain. They were interpreted as gram positive and gram negative organisms and biochemical panels were put accordingly, usually oxidase, catalase, coagulase, indole, citrate, methyl-red (MR), voges-proskure (VP), triple sugar iron(TSI),and urease. The organisms were identified and antimicrobial susceptibility test was performed.

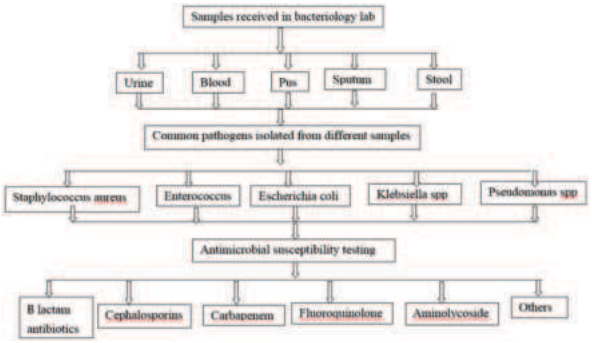
Antibiotic Susceptibility Testing:

Staphylococcus aureus, Enterococcus spp, E. coli, Klebsiella spp and Pseudomonas spp were the five commonly isolated pathogens. Antimicrobial susceptibility testing was done by Kirby Bauer Disk diffusion method. Results were interpreted by measuring the zone of inhibition. Antimicrobials were used based on CLSI guideline 2024.

Data analysis:

AST pattern of various pathogens were documented in XL-sheet. Categorical variables were reported as frequency (percentage). All analyses were performed using the Statistical Package for Social Science (SPSS) version 24.

SUMMARY OF METHODOLOGY:-



RESULT:

A total of 1609 samples received on lab were culture positive. Among them 64.2% (1033/1609) were urine, 27.65% (445/1609) pus, 6.02% (97/1609) blood and 1.05% (17/1609) were sputum and stool each.(Fig1)

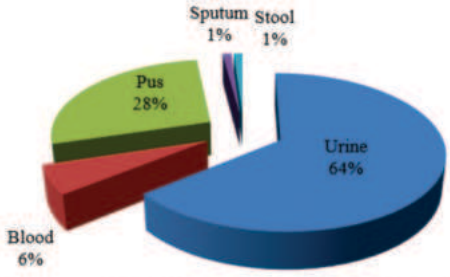


Fig 1: Frequency distribution of samples with isolated pathogens

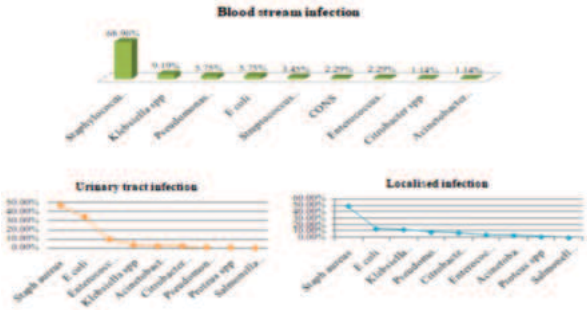
Among 1414 culture positive samples, various pathogens isolated were *Staphylococcus aureus* (48.72%), *Escherichia coli* (25.95%), *Enterococcus spp.* (7.63%), *Klebsiella spp.* (6.64%), *Citrobacter spp.* (3.74%), *Pseudomonas spp.* (3.46%), *Acinetobacter spp.* (2.54%), *Proteus spp.* (0.78%), *Streptococcus spp.* (0.22%), *Salmonella spp.* & CONS (0.14% each) (Table 1).

Table1: Frequency distribution of isolated pathogens (n=1414)

Organisms	Frequency	Proportion
Staph aureus	689	48.72%
E coli	367	25.95%
Enterococcus spp	108	7.63%
Klebsiella spp	94	6.64%
Citrobacter spp	53	3.74%
Pseudomonas spp	49	3.46%
Acinetobacter spp	36	2.54%
Proteus spp	11	0.78%
Streptococcus spp	3	0.22%
CONS	2	0.14%
Salmonella spp	2	0.14%

Commonly isolated pathogens in BSI were Staphylococcus aureus (68.96%),Klebsiella spp (9.19%), Pseudomonas spp(5.75%), E coli (5.75%), Streptococcus spp (3.45%), Enterococcus spp (2.29%), CONS (2.29%), Citrobacter spp (1.14%), Acinetobacter spp (1.14%).

In UTI we got Staphylococcus aureus (47.03%), E coli (34.25%), Enterococcus spp (9.7%), Klebsiella spp (3.42%), Acinetobacter spp (2.16%), Citrobacter spp(2%), Pseudomonas spp (0.68%), Proteus spp (0.57%), Salmonella spp (0.11%).In localised tissue infections at different sites we had Staph aureus (48.12%), E coli(13.74%), Klebsiella spp (12.41%), Pseudomonas spp(8.43%), Citrobacter spp (7.54%), Enterococcus spp (4.65%), Acinetobacter spp (3.54%), Proteus spp (1.33%) and Salmonella spp (0.22%). (Fig 2)



Antimicrobial sensitivity and resistant pattern of various pathogens isolated from different samples were given in table 2&3.

Table 2: Antimicroial Susceptibility Pattern

Class/mechanism	Antimicrobials	Staphylococcus aureus (n= 605)		Enterococcus spp (n=83)	
		Sensitive	Resistance	Sensitive	Resistance
B-lactam antibiotics	Penicillin	11(1.82%)	473(78.18%)	02(2.41%)	56(67.47%)
	Ampicillin	60(9.92%)	21(3.47%)	21(25.3%)	58(69.87%)
	Amoxyclav		01(0.1%)		
	Ticarcillin			14(16.86%)	12(14.46%)
Cephalosporins	Cefazolin	02(0.33%)	05(0.83%)		
	Cefoxitin	143(23.64%)	457(75.54%)	12(14.46%)	08(9.64%)
	Ceftriazone		03(0.49%)		
	Ceftazidime		01(0.1%)		
	Cefepime	01(0.1%)	01(0.1%)		
Aminoglycosides	Gentamycin	511(84.46%)	84(13.88%)	51(61.45%)	19(22.89%)
	Amikacin	01(0.1%)			
	Tobramycin		01(0.1%)		
	Streptomycin	01(0.1%)	113(18.68%)	18(21.68%)	59(71.08%)
	Clindamycin	358(59.17%)	240(39.67%)	06 (7.23%)	02(2.41%)
Fluorouinolones	Ciprofloxacin	389(64.29%)	180(29.75%)	39(46.98%)	25(30.12%)
	Levofloxacin	302(49.92%)	172(28.43%)	27(32.53%)	26(31.32%)
Glycopeptides	Vancomycin	468(77.35%)	134(22.15%)	38(45.78%)	05(6.02%)

Carbapenem	Imipenem		01(0.1%)	18(21.68%)	02(2.40%)
	Meropenem	94(15.54%)	22(3.63%)	21(25.30%)	05(6.02%)
	Doripenem	01(0.1%)			
Tetracyclines	Doxycycline	08(1.32%)	01(0.1%)		
	Tetracycline	354(58.52%)	59(9.75%)	03(3.61%)	01(1.2%)
Sulfonamides & trimethoprim	Cotrimoxazole	444(73.38%)	156(25.78%)	16(19.27%)	04(4.82%)
Poly myxins	PolymyxinB	102(16.86%)	12(1.98%)	24(28.91%)	01(1.20%)
	Colistin	01(0.1%)			
Macrolids	Erythromycin	237(39.17%)	358(59.17%)	15(18.07%)	05(6.02%)
Linezolids	Linezolid	480(79.34%)	113(18.67%)	68(81.92%) 68(81.92%) 68(81.92%)	16(19.27%)
	Nitrofurantoins	322(53.23%)	44(7.28%)	37(44.57%)	05(6.02%)

Table 3: Antimicrobial Susceptibility Testing

		Escherichia coli (n= 289)		Pseudomonas (n=61)		Klebsiella sp (n=142)	
Class	Antimicrobials	Sensitive (SN)	Resistance(R)	SN	R	SN	R
B-lactam antibiotics	Penicillin	80(27.68%)	33(11.42%)	9(14.75%)	4(6.56%)	29(20.42%)	37(26.06%)
	Ampicillin	42(14.53%)	246(85.12%)	2(3.28%)	1(1.64%)	17(11.97%)	125(88.02%)
	Amoxycillin	2(0.69%)	11(3.8%)			1(0.7%)	8(5.6%)
	Amoxyclav	76(26.29%)	186(64.35%)	2(3.28%)	1(1.64%)	47(33.09%)	78(54.92%)
Cephalosporins	Cefazolin	82(28.37%)	201(69.55%)	2(3.28%)	2(3.28%)	38(26.76%)	101(71.13%)
	Ceftriazone	134(46.36%)	146(50.51%)	2(3.28%)	1(1.64%)	69(48.59%)	73(51.40%)
	Ceftazidime	6(2.07%)	7(2.42%)	3(4.92%)	53(86.88%)	2(1.41%)	1(0.7%)
	Cefepime	145(50.17%)	140(48.44%)	19(31.15%)	42(68.85%)	65(45.77%)	75(52.82%)
Carbapenems	Imipenem	139(48.09%)	58(20.06%)	19(31.15%)	26(42.62%)	68(47.88%)	36(25.35%)
	Meropenem	192(66.43%)	43(14.88%)	39(63.93%)	9(14.75%)	94(66.19%)	33(23.24%)
	Doripenem	272(94.12%)	15(5.19%)	53(86.88%)	7(11.47%)	134(94.37%)	11(7.75%)
Fluoroquinolones	Ciprofloxacin	159(55.01%)	91(31.48%)	51(83.6%)	9(14.75%)	104(73.23%)	35(24.64%)
	Levofloxacin	145(50.17%)	81(28.03%)	43(70.49%)	18(29.51%)	77(54.23%)	41(28.87%)
Aminoglycosides	Gentamycin	243(84.08%)	44(15.22%)	40(65.57%)	8(13.11%)	102(71.83%)	21(14.78%)
	Amikacin	179(61.93%)	24(8.3%)	46(75.4%)	8(13.11%)	86(60.56%)	13(9.15%)
	Tobramycin	162(56.05%)	39(13.49%)	39(63.93%)	9(14.75%)	77(54.23%)	29(20.43%)
	Streptomycin	08(2.77%)	14(4.84%)				4(2.82%)
	Clindamycin	02(0.69%)					
Aztreonam	Aztreonam			35(57.38%)	16(26.23%)		
Linezolid	Linezolid	53(18.34%)	64(22.15%)	09(14.75%)	04(6.56%)	28(19.72%)	36(25.35%)
Glycopeptides	Vancomycin	74(25.61%)	39(13.49%)	3(4.92%)	1(1.64%)	40(28.16%)	14(9.86%)
Tetracyclines	Tetracycline	151(52.25%)	70(24.22%)	8(13.11%)	5(8.19%)	86(60.56%)	33(23.24%)
Sulfonamides & trimethoprim	Cotrimoxazole	166(57.44%)	96(33.22%)	2(3.28%)	1(1.64%)	89(62.67%)	51(35.92%)
	Nitrofurantoin	217(75.08%)	23(7.96%)	17(27.87%)	17(27.87%)	77(54.22%)	22(15.49%)
	Piperacillin-tazobactum	152(52.59%)	34(11.76%)	35(57.37%)	10(16.39%)	76(53.52%)	26(18.31%)
	PolymyxinB	67(23.18%)	08(2.77%)	4(6.56%)	8(13.11%)	27(19.01%)	11(7.75%)

DISCUSSION:

Use of irregular, low dose antibiotics is critical to manage infections as well as brining resistant to the antibiotic for an individual inspite of get rid of infection of individual over & above resistance of co-infection to surrounding individuals. Regular assessment of antibiotic use is necessary to preserve its sensitivity. Hospital based studies showed higher and varied spectrum of resistance in different regions. Gap of public health monitoring is to be ensured to overcome the resistance of any antibiotic.[8]

A cross-sectional study with urine, blood, sputum, pus, and stool sample in microbiology lab of TRIHMS was done.Urine (64.2%) being the commonest sample followed by pus (27.65%) & blood (6.02%).Urinary tract infection (UTI), blood stream infection (BSI) are public health problem caused by a range of pathogens but most commonly by Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis, Staphylococcus aureus.[9] In our study also these were the commonest specimen with decreasing order of their frequency as Staphylococcus aureus (47.03%), E coli (34.25%), Enterococcus spp (9.7%), Klebsiella spp (3.42%)in UTI; Staphylococcus aureus (68.96%),Klebsiella spp (9.19%), Pseudomonas spp(5.75%), E coli (5.75%) in BSI, and Staph aureus (48.12%), E coli(13.74%), Klebsiella spp (12.41%), Pseudomonas spp(8.43%), in localised infections.

Staphylococcus being the commonest isolated pathogen, showed resistance to different antimicrobials. Talapan D et al in their study showed the resistance of staphylococcus to oxacillin (39.11%), erythromycin (49.97%), clindamycin (36.06%), ciprofloxacin (9.98%), rifampicin (5.38%), gentamycin(5.95%),trimethoprim-sulfamethoxazole (0.96%) respectively. [10] Nwankwo E O et al in their study showed that staph aureus is sensitive to Gentamicin, Amoxycillin/ clavulanate, Streptomycin, Cloxacillin, Erythromycin, Chloramphenicol, Cotrimoxazole, Tetracycline, Penicillin, Ciprofloxacin, Ofloxacin, Levofloxacin, Ceftriaxone, Amoxycillin, vancomycin, levofloxacin, ofloxacin in 92.4%, 63.0%, 44.2%, 35.8%, 52.4%, 61.9%, 15.5%, 31.2%, 7.1%, 78.9%, 76.6%, 100%, 71.4%, 30.7% and 100% ,93.7% and 68.7% of staph respectively.[11] Our study showed, highest sensitivity of staphylococcus aureus with Gentamycin (84.46%), Linezolid (79.34%), Vancomycin(77.35%), Cotrimoxazole (73.38%), Ciprofloxacin (64.29%) and highest resistance to cefoxitin (75.54%) penicillin (78.18%), erythromycin (59.17%), clindamycin (39.67%).

Rudy M et al in their study showed that E. faecium were sensitive to glycopeptides like vancomycin, teicoplanin (100%), penicillin (32%), tetracycline (19%), ciprofloxacin (14%) and nitrofurantoin(50%) and aminoglycosides in 17% of E. faecalis and 29% of E. faecium.[12] Our study showed highest frequency of sensitivity in Enterococcus spp with Linezolid (81.92%), Gentamycin (61.45%), Ciprofloxacin (46.98%),

Vancomycin (45.78%), Nitrofurantoin (44.57%) and highest resistance was observed with Streptomycin (71.08%), Ampicillin (69.87%) and penicillin (67.47%).

Kibert M et al showed high resistance rates of E coli to erythromycin (89.4%), amoxicillin (86.0%),tetracycline (72.6%) and significantly high sensitivity to nitrofurantoin (96.4%), norflaxocin (90.6%), gentamicin (79.6%) and ciprofloxacin.[13] Our study showed highest frequency of sensitivity in E coli with Doripenem (94.12%), Gentamycin (84.08%), Nitrofurantoin (75.08%), Meropenem (66.43%), Amikacin (61.93%), Tobramycin (56.05%) and resistance with Ampicillin (85.12%), Cefazolin (69.55%), Amoxyclav (64.35%).

Lin W P et al showed susceptibility of ESBL producing Klebsiella spp to amikacin(64.8%), ciprofloxacin(33.3%), ertapenem(57.1%),imipenem(63%),cefotaxime(81.2%).[14] Our study showed susceptibility of Klebsiella spp to Doripenem (94.37%), Ciprofloxacin (73.23%), Gentamycin (71.83%), Meropenem(66.19%) and resistant to Ampicillin(88.02%), Cefazolin (71.13%), Amoxyclav (54.92%), Cefepime (52.82%).

Viren A. J et al showed susceptibility of pseudomonas spp to aminoglycosides (32-48%), fluoroquinolones (26-37%), imipenem (78.57%), meropenem (69.64%), aztreonem (71.43%) and resistant to tetracyclines, macrolides and chloramphenicol in 75and 91%[15] Our study showed susceptibility of pseudomonas spp to Doripenem (86.88%), Ciprofloxacin (83.6%), Amikacin (75.4%), Levofloxacin (70.49%), Gentamycin (65.75%), Meropenem & Tobramycin (63.93%), and resistant to ceftazidime (86.88%) & cefepime (68.85%).

CONCLUSION:

This study provided comprehensive systemic assessment of antimicrobial spectrum based on various tests in our laboratory showing commonest specimen being urine and commonest pathogen being staphylococcus aureus. Resistance in highest quantum showed in penicillin, ampicillin, cefoxitin, clindamycin, erythromycin, streptomycin, ticarcillin in gram positive isolates and ampicillin, amoxyclav, cefazolin, ceftazidime, cefepime in gram negative isolates. Microbiologists should properly monitor the choice of antibiotics to be used in treatment to achieve maximum clinical outcome of efficacy.

Ethical Statement:

Study was approved by Institutional Ethics Committee on 29th August, 2023 with memo no:TRIHMS/ETHICS/01/2019-20/56.

Conflict of Interest: None.

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