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Isolation, Identification and Antimicrobial Sensitivity of Bacterial Isolates from Pus, Sputum and Urine Samples

KEYWORDS: Body fluid culture, Kirby-Bauer method, Anti biogram

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ABSTRACT *A total of 8 different microbial organisms (7 bacteria and 1 Candida species) were isolated from 69 positive samples out of 155 clinical samples specimens of pus, urine, sputum and other body fluid culture collected from public health Civil Hospital Vyara.The most common isolates observed in our study were Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, S.pyrogens, Proteus ,Enterococcus spp, and Candida species. Staphylococcus aureus showed highest sensitivity towards Linezolid (92.3%). Streptococcus pyrogenes was found to be 100 % sensitive towards Vancomycin, Cefuroxzime, Cefazoline,Teicoplanin and 100% resistance towards Erythromycin,Ofloxacin, Ciprofloxacin, Cefadroxil, Gentamycin, Ampicillin + Sulbactam, Cloxacillin/ Oxacillin, Levofloxacin, Cefdinir and Linezolid. Enterococcus showed 100% sensitivity towards Ampicillin + Sulbactam and Moxifloxacin and moderate towards the rest of the antibiotics. E.coli was 90 % sensitive to most of the antibiotics. Pseudomonas Spp showed 100% sensitivity towards Ceftriaxone, Gentamycin,Piperacillin + Tazobactam. Klebsiella Spp showed highest sensitivity (89.5%) towards Ciprofloxacin.*

Introduction

Infectious diseases are a major cause of death around the world and are responsible for affecting the living conditions of millions of people. Microbes (bacteria, fungi, parasites and viruses) cause infectious diseases and antimicrobial agents (such as penicillin, streptomycin and other antimicrobial) have been developed to combat the severity and spread of many of these diseases. The use of antimicrobial agents for prevention or treatment of infections in humans in any dose and over any time period cause a reduction in microbial populations. The emergence of resistance to antimicrobial in previously susceptible bacterial pathogens is a major challenge to infectious disease.

Antibiotics were considered to be the most effective therapeutic agents to combat microbial infections. But due to the overuse of antibiotics, an emergence and spread of multidrug resistant strains among different groups of microorganisms have taken place. Infections resistant bacteria are emerging threat all over the world both as hospital acquired as well as community acquired microorganisms

The antimicrobial resistance is one of the main problems in clinical as well as public health view points. The antimicrobial resistance is not only increasing morbidity and mortality but also great economic loss encompassing use of more expensive antibiotics to treat infection as well as threat of resistance to them.[1] When immunity is decreased or humans are attacked by virulent bacteria resulting in respiratory tract infection, urinary tract infection and gastrointestinal infection. Antimicrobials like third generation cephalosporines are used to cure these infections. These drugs are highly active against gram-negative cocci, gram-negative bacilli and anaerobes. Hence, there is a need to conduct area specific infections and their resistance patterns, so as to generate data that would help clinicians to choose the correct therapy. Antimicrobial resistance in *E. coli* has been reported worldwide and increasing rates of resistance among *E. coli* is a growing concern in both developed and developing countries. A rise in bacterial resistance to antibiotics complicates treatment of infections.[2]

Recently, WHO warned the community that Multi-drug resistant bacteria are emerging worldwide, which is a big challenge to healthcare and if immediate action is not taken, then antibiotics may lose their power to cure diseases. In India, the reasons for development of antimicrobial resistance could be due to irrational use of antibiotics, over the counter availability of higher or broader antimicrobial agents, higher prevalence of infection and poor monitoring of antibiotic susceptibility in hospitals.[3]

Drug resistance among gram negative bacilli is of clinically importance and pose serious threat to public health. Numerous studies have been performed to identify susceptibility patterns of gram negative bacterial isolates.

The present study was done to isolate,identify and determine the sensitivity of the isolates fro different clinical samples of Civil Hospital,Vyara.

MATERIALS and METHODS

Sample collection

A total number of 155 Pus, Sputum,Urine and other body fluid samples were included in the study.The above samples were taken from patients who came for treatment to Civil Hospital Vyara. All clinical samples were collected and processed according to standard operating procedures.

Isolation and Identification of pathogenic isolates

Clinical isolates were identified by their morphological characteristics on MacConkey agar, Blood agar medium and Nutrient agar. The colonies obtained on the respective media were processed for Gram Staining. The biochemical tests performed were **C**atalase test, Coagulase test, Indole test, Oxidase test, Citrate Test and Urease test

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Antibiotic susceptibility tests

The identified isolates were then subjected to sensitivity test on Muller Hinton Agar The using the Kirby-Bauer disk diffusion method.[4] The commercial available antibiotic discs used for the study were **A**mikacin, Amoxycillin, Ampicillin, Azithromycin**,**Amoxicillin + Clav, Ampicillin + Sulbactam, bacitracin, Ciprofloxacin, Cefixime,Cefadoxyl, Cefotoxamine, Cefalaxine, Cetriaxone, Cefepime, Cefazoline, Cefuroxzime, Cefdinir, Cefalaxine, Ceftazidime, Cloxacillin / Oxacillin, Erythromycin, Gentamycin, Imipenem, Levofloxin, Linezoid, Moxifloxacin, Norfloxacin, Nitrofurantoin, Optochin, Ofloxacin, Penicillin G, Piperacillin+Tazobactam,Trim ethoprim+Sulfa,Teicoplanin,Tetracycline,Vancomycin.

Result and Discussion

Collection of sample, isolation and identification of pathogenic bacteria and yeasts from different clinical samples and specimens:-

Out of the 155 specimens, 69 (44.51%) specimens were found to have microbial pathogens. A total of 8 different microbial organisms (7 bacteria and 1Candida species) were isolated in 69 specimens.(Table No:1 and Table No:2)

Table No1: Number and Frequency of positive and negative cases for microbial growth from different clinical samples and specimens

Sr.No	Source of samples and specimens	Total	No of positive Cases	%	No of Negative Cases
1	Body Fluid Urine Samples	20	9	13.04	11
2	Pus (wound) swarb	40	31	44.93	9
3	Respiratory tract: Sputum	48	22	31.88	26
4	Other body fluid	47	7	10.14	40
		155	69	100	86

The most common isolates observed in our study were *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae*, *S.pyrogens,Proteus ,Enterococcus spp,* and Candida species.The most frequent isolate in our study was *Escherichia coli*, which was isolated in 28.98% of the culture specimens, while in other studies it was isolated in 47.4%9, 41.7% and 32.39%.[5,6] 80 % of E.coli were present singly and 25 % showed mixed growth.The next predominant isolate found was *Klebsiella pneumoniae* (27.53%). 70 % of *Klebesialla* were present singly and 30 % were showing mixed growth. *P.aeruginosa* was present *in 8.69 %* whereas *P.vulgaris* was found in 2.08 % of the total positive samples. Gram negative bacilli dominance in the aerobic growth in pus has been highly recorded.[7,8]

Among the Gram Positive bacteria the dominant isolate was *Staphylococcus.aureus* i.e 18.84 % out of which 85% of the isolate were present singly and 15% in mixed growth. *S.pyogen*s and *Enterococcus spp* were present in 2.89% and 5.79 % of the total positive samples. Candida albicans yeast was also found in 2.89 % positive samples. Our observation very well coincide with the works reported by various authors across the country. Aerobic bacteria and gram positive *S.aureus* was found to be the most commonly occurring pathogen [9]whereas second most common isolate after pseudomonas spp.[10]

Table No2: Isolation of bacterial isolates

Types of isolate	Urine	Respiratory Tract	Pus	Blood and other fluid
E.coli	4	1	14	1
K.pneumoniae	0	15	3	1
P.aeruginosa	0	3	3	0
P.vulgaris	0	0	2	0
S.aureus	4	0	7	2
S.pyrogens	0	0	0	2
Enterococcus spp	0	2	2	0
Candida spp	1	1	0	1
	9	22	31	7

In this study the isolated pathogenic bacteria and yeast were identified on the bases of their morphological characteristics and the gram stains reaction to differentiate them to gram-negative and gram-positive bacteria, and yeast

Table No3: Biochemical Test

Sr.No	Biochemical Test	E.coli	S. aureus	P. vulgaris	S. pyrogens	***Enterococcus spp.***	P. aeruginosa	Klebsiella pneumoniae
1	Catalase test	+	+	+	-	-	+	+
2	Coagulase test	-	+	-	-	-	-	-
3	Oxidase test	-	-	-	-	-	+	-
4	MR	+	+	-	-	-	-	-
5	VP	-	+	-	+	+	-	+
6	Nitrate	+	+	+	-	-	-	+
7	Indole test	+	-	+	-	-	-	-
8	Citrate test	-	+	-	-	-	+	+
9	Urease test	-	+	+	-	-	+	+

Antimicrobial susceptibility

Antibiogram revealed that gram positive cocci*S taphylococcus aureus* showed highest sensitivity towards Linezolid (92.3%), 84.6 % sensitivity towards Cefadroxil,Erythromycin and 76.9% sensitivity towards Azithromycin, Gentamycin and Ampicillin + Sulbactam Whereas 76.9 % resistance towards Amoxicillin and Cefuroxzime.*Streptococcus pyrogenes* was found to be 100 % sensitive towards Vancomycin, Cefurox zime,Cefazoline,Teicoplanin and 100% resistance towards Erythromycin,Ofloxacin, Ciprofloxacin, Cefadroxil, Gentamycin, Ampicillin + Sulbactam, Cloxacillin/ Oxacillin, Levofloxacin, Cefdinir and Linezolid. *Enterococcus showed 100% sensitivity towards* Ampicillin + Sulbactam and Moxifloxacin and moderate towards the rest of the antibiotics.

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The dominant gram negative bacteria E.coli was 90 % sensitive to most of the antibiotics. *Pseudomonas Spp* showed 100% sensitivity towards Ceftriaxone, Gentamycin,Piperacillin + Tazobactam and 83.3 % resistance to Cefixime. *Klebsiella Spp* showed highest sensitivity (89.5%) towards Ciprofloxacin and 89.5 % resistance to Cefixime wheras moderate sensitivity and resistance towards the other antibiotics. *P.vulgaris* showed sensitivity towards Amikacin Ciprofloxacin, Cefixime ,Ceftazidime,Cefazoline and Ofloxacin whereas resistance towards the rest of the antibiotics

Table No 4:Antibiotic Susceptibility Test of Gram Positive Bacteria

Sr.No	Antibiotic	*Staphylococcus aureus* (n=13)		*Streptococcus pyrogenes* (n=2)		*Enterococcus* (n=13)	
		Sensitivity	Resistant	Sensitivity	Resistant	Sensitivity	Resistant
1	AMX	23.1	76.9	50	50	50	50
2	AMP	53.9	46.1	50	50	50	50
3	AZM	76.9	23.1	50	50	25	75
4	E	84.6	15.4	0	100	75	25
5	OF	61.5	38.5	0	100	50	50
6	CIP	69.2	30.8	0	100	75	25
7	CFR	84.6	15.4	0	100	75	25
8	CPM	38.5	61.5	0	100	25	75
9	GEN	76.9	23.1	0	100	25	75
10	AMC	38.5	61.5	50	0	75	25
11	A/S	76.9	23.1	0	100	100	0
12	AK	61.5	38.5	50	50	25	75
13	VA	61.5	38.5	100	0	25	75
14	OX	38.5	61.5	0	100	50	50
15	CZ	69.2	30.8	100	0	50	50
16	CXM	23.1	76.9	100	0	25	75
17	TE	61.5	38.5	50	50	75	25
18	CDR	46.2	53.8	0	100	25	75
19	TEI	46.2	53.8	100	0	50	50
20	LZ	92.3	7.7	0	100	50	50
21	MO	69.2	30.8	50	50	100	0
22	P	76.9	23.1	100	0	75	25
23	LE	69.2	30.8	0	100	75	25

Table No 5: Antibiotic Susceptibility Test of Gram Negative Bacteria

Sr. No	Antibiotic	*E.coli* (n=20)		*Pseudomonas Spp* (n=6)		*Klebsiella Spp* (n=19)		*P.vulgaris* (n=2)	
		Sensitivity	Resistant	Sensitivity	Resistant	Sensitivity	Resistant	Sensitivity	Resistant
1	AK	90	10	50	50	31.6	68.4	100	0
2	AMX	90	10	33.3	66.7	26.3	73.7	0	100
3	AMP	90	10	83.3	16.7	52.6	47.4	0	100
4	A/S	90	10	83.3	16.7	31.6	68.4	0	100
5	CIP	55	45	33.3	66.7	89.5	10.5	100	0
6	CFM	90	10	16.7	83.3	10.5	89.5	100	0
7	CTX	85	15	83.3	16.7	68.4	31.6	0	100
8	CN	90	10	83.3	16.7	26.3	73.7	0	100
9	CTR	90	10	100	0	57.9	42.1	0	100
10	CZ	90	10	83.3	16.7	26.3	73.7	100	0
11	CAZ	70	30	83.3	16.7	73.7	26.3	100	0
12	GEN	90	10	100	0	42.1	57.9	0	100
13	IPM	95	5	100	0	57.9	42.1	0	100
14	LE	90	10	50	50	26.3	73.7	0	100
15	OF	90	10	33.3	66.7	78.9	21.1	100	0
161	PIT	90	10	100	0	57.9	42.1	0	100
71	TE	60	40	66.7	33.3	63.2	76.8	50	50
18	CPM	40	60	33.3	66.7	42.1	57.9	0	100

DISCUSSION

Out of the 155 specimens, 69 (44.51%) specimens were found to have microbial pathogens. A total of 8 different microbial organisms (7 bacteria and 1 Candida species) were isolated in 69 specimens. The most common isolates observed in our study were *Escherichia coli*, *Staphylococcus aureus,Pseudomonas aeruginosa, Klebsiella pneumoniae, S.pyrogens, Proteus , Enterococcus spp,* and *Candida species. Escherichia coli,* was isolated in 28.98% of the culture specimens,The next predominant isolate found was *Klebsiella pneumoniae* (27.53%). *Staphylococcus.aureus* i.e 18.84.

All the isolates displayed variable sensitivity to the antibiotics tested. *Staphylococcus aureus* showed highest sensitivity towards Linezolid (92.3%),which was very similar to the results obtained by[11]. *Staphylococcus aureus* isolates, sensitivity to linezolid (30mcg) was 87.71% .*Enterococcus* showed 100% sensitivity towards Ampicillin + Sulbactam and Moxifloxacin.

Streptococcus pyrogenes was found to be 100 % sensitive towards Vancomycin, Cefuroxime,Cefazoline,Teicoplanin and 100% resistance towards Erythromycin, Ofloxacin, Ciprofloxacin, Cefadroxil, Gentamycin, Ampicillin + Sulbactam, Cloxacillin/ Oxacillin, Levofloxacin, Cefdinir and Linezolid.

E.coli was 90 % sensitive to most of the antibiotics. All E. coli strains isolated from surgical wounds were sensitive to amikacin, gentamicin, cefoperazone, ceftriaxone, imipenem and ciprofloxacin[12]. *Pseudomonas Spp* showed 100% sensitivity towards Ceftriaxone, Gentamycin,Piperacillin + Tazobactam. *P. aeruginosa* were more sensitive to combination drugs like piperacillin+tazobactum (93.5%) and cefoperazone+sulbactum (92.3%) followed by imipenem (88.2%), meropenem (87.1%).[13] Sensitivity to amikacin, tobramycin, gentamicin and ceftazidime ranges from 35% to 55%.*Klebsiella Spp* showed highest sensitivity (89.5%) towards Ciprofloxacin and 89.5 % resistance to Cefixime.*Klebsiella Spp* against various antibiotics tested also showed nearly similar results.[14].*P.vulgaris* showed sensitivity towards Amikacin, Ciprofloxacin, cefixime, Ceftazidime,Cefazoline and Ofloxacin whereas resistance towards the rest of the antibiotics. *Proteus* isolates were highly susceptible to Cefotaxime, Ofloxacin, Gentamycin, Amikacin, Lomefloxacin, Ciprofloxacin and Cefaperazone. [15]

CONCLUSIONS

There is a need to pay attention to microbial sensitivity and resistance pattern to various antimicrobials. *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, S.pyrogens,Proteus ,Enterococcus spp,* and Candida species isolated showed variable sensitivity towards the antibiotics tested.

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