

# Identification and Characterization of Aerobic Bacteria Isolated from Normally Sterile Body Sites of Patients Attending a Tertiary Care Hospital in Ajmer, India

Dr. Neelam Kholiya \*, Dr. B. L. Dariya \*\*, Dr. Vijaylatha Rastogi \*\*\*, Dr. Geeta Parihar \*\*\*\*

\* &\*\*\* &\*\*\*\* Microbiology, JLN Medical College, Ajmer  
\*\* Biochemistry, SMS Medical College, Jaipur

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**Abstract-** The widespread use of antibiotics has led to the emergence of antimicrobial pathogens that contribute to illness and death. This study was aimed at examining the differentiation of bacterial pathogens and their drug susceptibility patterns from normally sterile body sites of Patients from inpatients and outpatients attending a tertiary care centre.

**Methods and Material:** A cross sectional study was conducted at JLN Hospital Ajmer from January to June, 2017. Samples were collected from each study participant and inoculated into appropriate media. The bacterial pathogens were identified using standard microbiological methods. Antimicrobial susceptibility tests were performed using disk diffusion technique following Modified Kirby-Bauer method.

**Results:** A total of 250 study subjects were included in the study with bacterial isolation rate of 69 (18.75%). Of all, 151 (60.8%) were males. Thirty eight (55%) of the isolates were Gram-negative, 29 (42%) were Gram-positive and 2 (3%) were BYC (Budding Yeast Cell). From the total isolates, Klebsiella species were the most predominant isolate 28/69 (28.98%) followed by Coagulase Negative Staphylococcus (24.64%) and Pseudomonas species (21.74%). Imipenem (93%) was found to be most effective drug against the Klebsiella species followed by amikacin (90%). While cefoperazone, ampicillin, ceftriaxone were least effective drugs against Klebsiella species.

Imipenem (87%) was also the most effective drug against the Pseudomonas species. Moderately effective drugs were ciprofloxacin (73%), amikacin (60%) and piperacillin (47%). Least effective were ampicillin (13%) and ceftriaxone (13%). It was seen that vancomycin and linezolid were the most effective drugs for Gram positive organisms. Least sensitive drugs were cotrimoxazole, erythromycin and ampicillin. Penicillin showed 100% resistance.

**Conclusion:** High prevalence of bacterial isolates was found, Klebsiella species being the most dominant. High rates of multiple drug resistance pathogens to the commonly used antimicrobial agents were isolated. Therefore, concerned bodies should properly monitor the choice of antibiotics to be used as prophylaxis and empiric treatment in the study area.

**Index Terms-** Normally Sterile Body Sites, Aerobic Bacteria, antimicrobial susceptibility.

## I. INTRODUCTION

Sterile sites are those in which no bacteria or microbes exist as commensals when in a healthy state, such that any growth is considered significant and can either be pathogenic micro-organisms or contaminants. On the contrary, for non-sterile sites, not all isolated organisms are significant as they can be normal flora.

Diagnosis of sterile site infections is based on culture of properly collected and processed samples. Preliminary reports such as gram staining, biochemical analysis may be helpful in providing immediate information to support the diagnosis and justify initiation of antibiotic treatment. However since definitive diagnosis is based on quantitative cultures, the course of antibiotic therapy should be determined after the culture results have been confirmed. Unfortunately in most instances it is either not possible to wait for culture reports hence empiric treatment is commenced, or laboratory facilities are unavailable or unreliable.

Infections caused by drug resistant organisms are difficult to treat hence limiting the therapeutic options for treatment. With growing antimicrobial resistance in India, reliance on international guidelines is insufficient. There is need of research such as this one to get antibiotic susceptibility testing profiles for pathogens from sterile site infections in our local setting, to enable us formulate local guidelines for treatment.

This study was done for identifying the bacterial pathogens and their antimicrobial susceptibility pattern in the patients admitted in a tertiary care Hospital, Ajmer. Any microorganism isolated from normally sterile body site should be considered a pathogen.

### Subjects and Methods:

This study was done at Department of Microbiology, along with various inpatient and outpatient departments of J.L.N. Medical College and attached Hospitals Ajmer city, Rajasthan, India. Ethical approval prior to conducting the research was obtained from JLN Medical College & Hospital, Ajmer, Ethics and Research Committee. Permission to extract data from the hospital registers and records was obtained from the Microbiology Laboratory Office. The study was a minimal risk study as there was no direct patient involvement but a retrospective review of the records. This was a hospital based cross-sectional study conducted from January 2017 to June 2017. Data analysed from available data of the patients' laboratory records at Microbiology department of J. L. N. Medical College, Ajmer. Samples were collected from both IPD & OPD department of J. L. N. Medical College, Ajmer. A total of 250 consecutive samples from sterile body site were collected for culture at microbiology laboratory, J. L. N. Medical College, Ajmer. Out of them 69 Samples were found to be culture positive, rest were sterile or no growth was seen. Any growth obtained was identified by using standard microbial procedure by colony characteristics, gram staining, hanging drop, motility testing and standard biochemical test. The bottles were evaluated visually for any change in the appearance for hemolysis, turbidity and gas formation. The Gram staining from the broth and culture smear were compared for the presence of similar organisms. Antibiotic Sensitivity Testing was done by Modified Kirby Bauer Method.

**Results:**

A total of 250 samples from different sterile body sites received at Microbiology Laboratory, J. L. N. Medical College & Hospital, Ajmer, from January 2017 to June 2017. Out of 250 samples 247(98.8%) of body fluids were from IPD, rest of all from OPD. Out of 250 samples collected 152 (60.8%) were from male patients and 98(39.2%) were from female patient. Samples collected from patients of different groups viz. 84 (70.2%) < 20 years , 107 (16.9%) between 21-40 years, 43 (9.1%) between 41-60 years ,16 (3.2%) were > 61 years . Out of 250 samples 69 (28%) samples were culture positive while 181 (72%) were culture negative.

**Table: Sample Profile**

Samples	Total no. of samples	Growth	No growth
Blood	192	61	131
CSF	29	3	26
Pleural fluid	10	2	8
Ascitic fluid	14	1	13
Liver Abscess fluid	5	2	3
<b>Total</b>	<b>250</b>	<b>69</b>	<b>181</b>

In this study, a total of 69(27.6%) samples were growth positive isolated from 250 body fluids. Among all growth positive samples, 55% (38) were gram-negative bacteria, with 42% (29) were gram-positive bacteria along with 3% (2) BYC were isolated from these clinical samples.

Among the isolates Klebsiella found to be the most frequent isolates 20 (28.98 %) followed by Coagulase Negative Staphylococcus (CoNS) 17(24.64%), and Pseudomonas 15 (21.74%) were the most dominate isolates. Other isolates were Enterococcus 8 (11.59%), CoPS 4 (5.78%), and Escherichia coli 3 (4.35%). BYC were isolated in 2 (2.9%) of cases.

**Graph: Frequency and type of microorganism isolated.**

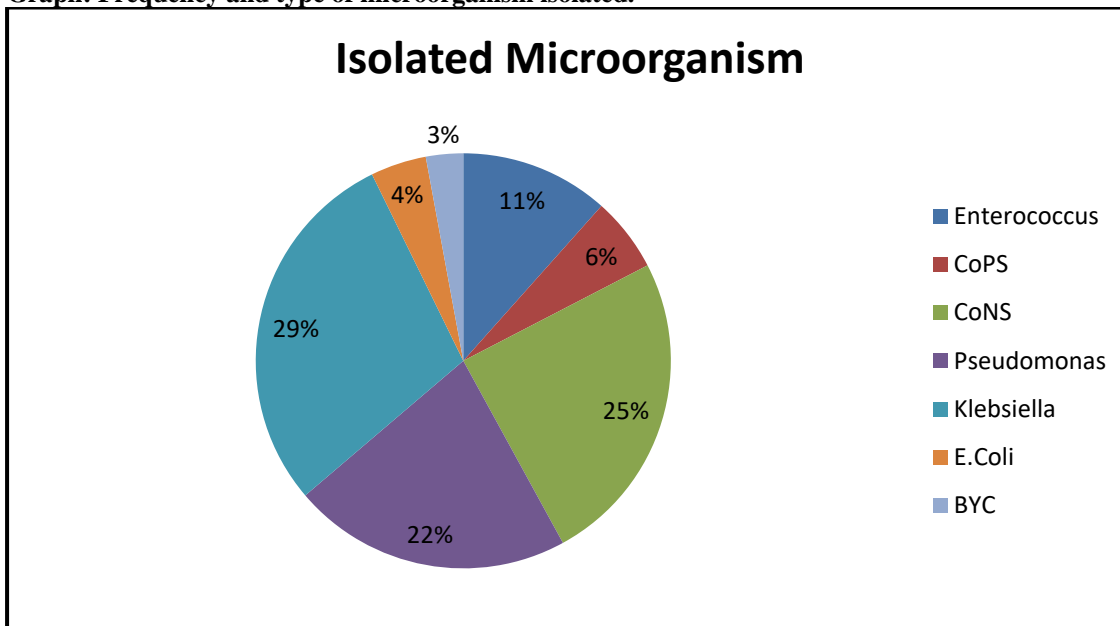


Table: Bacterial Isolation in Different Body Fluids

Microorganism isolated	Type of specimen					
	Blood	CSF	Pleural fluid	Ascitic fluids	Liver Abscess Fluid	Total
<i>Klebsiella spp.</i>	20(8)	0	0	0	0	20(8)
CoNS	17(6.8)	0	0	0	0	17(6.8)
<i>Pseudomonas spp.</i>	7(2.8)	3(1.2)	2(0.8)	1(0.4)	2(0.8)	15(6)
<i>Enterococcus spp.</i>	8(3.2)	0	0	0	0	8(3.2)
CoPS	4(1.6)	0	0	0	0	4(1.6)
<i>E. coli</i>	3(1.2)	0	0	0	0	3(1.2)
BYC	2(0.8)	0	0	0	0	2(0.8)
No growth	131(52.4)	26(10.4)	8(3.2)	13(5.2)	3(1.2)	181(72.4)
<b>Total</b>	192(76.8)	29(11.6)	10(4)	14(5.6)	5(2)	250

Vancomycin and Linezolid remained the most active drug in infections caused by Gram positive organisms, followed by amoxyclav, oxacillin & ciprofloxacin. The other drug found to be effective against Gram positive isolates include tetracycline & netilmycin. Penicillin & ampicillin were least effective. *Klebsiella* species show 90% sensitivity to imipenem followed by amikacin (85%) and tetracycline (60%). Imipenem showed 87% efficacy against the *Pseudomonas aeruginosa* followed by ciprofloxacin (73%), amikacin (60%) & piperacillin (47%).

**Antibiotic sensitivity pattern of Gram positive organism**

Isolate		AMP	P	AMC	CIP	TE	GEN	NET	E	LZ	OX	VA	COT	CX	CD
<i>Staphylococcus aureus</i> (n=4)	S	1	0	3	3	3	3	3	0	4	3	4	2	3	1
	R	3	4	1	1	1	1	1	4	0	1	0	2	1	3
	%	25	0	75	75	75	75	75	0	100	75	100	50	75	25
CoNS (n=17)	S	3	0	13	11	13	12	12	9	17	13	17	5	11	8
	R	14	17	4	6	4	5	5	8	0	4	0	12	6	9
	%	18	0	76	65	76	71	71	53	100	76	100	29	65	47
<i>Enterococcus</i> spices (n=8)	S	1	0	5	1	4	4	5	3	8	3	6	2	3	2
	R	7	8	3	7	4	4	3	5	0	5	2	6	5	6
	%	13	0	63	13	50	50	63	37	100	37	75	25	37	25

**Antibiotic sensitive pattern of Gram negative organisms**

Isolate		A M P	A M C	C I P	C T X	C A Z	C F M	C T R	C P Z	C P M	A T	I M P	T E	G E N	N E T	A K	P I
<i>Klebsiella spp.</i> (20)	S	0	12	12	10	10	10	6	0	12	13	18	12	12	17	-	
	R	20	8	8	10	10	10	14	20	8	7	2	8	8	8	3	
	%	0	60	60	50	50	50	30	0	60	65	90	60	60	85	-	
<i>P.aeruginosa</i> (15)	S	2	2	11	4	6	-	2	-	4	7	13	2	6	-	9	7
	R	13	13	4	11	9	-	13	-	11	8	2	13	9	-	6	8
	%	13	13	73	27	40	-	13	-	27	47	87	13	40	-	60	47
<i>E. coli</i>	S	0	0	0	0	0	0	0	0	0	0	3	0	0	0	3	-

(3)	R	3	3	3	3	3	3	3	3	3	3	0	3	3	3	0	-
	%	0	0	0	0	0	0	0	0	0	0	100	0	0	0	100	-

**Discussion:**

Coagulase negative staph. and Klebsiella spp. were the most prevalent isolates in our study, in agreement with study conducted Iran by Rezaeizade et al., in Iran by Babak A et al., in Korea by Yoon et al., in Brazil by Daur et al. The detection of Coagulase negative staph. & Klebsiella spp. may be the present of an opportunistic pathogen associated with nosocomial infections, due to poor infection control facilities.

Study done in Iran by Babak et al., in Nepal by Shrestha et al., reported Haemophilus influenza as most common organism while in our study no Haemophilus influenza was reported. Since, Hemophilus influenzae type b (Hib) once a common cause of meningitis has disappeared due to the effectiveness of the extended immunization program. The reason for this is not clear but may be due to the new vaccine have been giving as part of routine child immunization activities, which might have reduced the occurrence of invasive diseases due to H. influenzae.

In CSF P. aeruginosa is one of the most common nosocomial pathogens causing iatrogenic meningitis and has greater ability to develop resistance to virtually any antibiotic it's exposed to because of multiple resistance mechanisms that can be present concurrently within the pathogen (Livermore DM, 2001). In this study an incidence of 6% was found compared to that of 16% in two different studies by Nagaveni et al (2011) and Jones et al (1997). Although lower, this incidence is still high as this organism is mainly found in critical patients whose immunity is low.

In this study, the majority of isolates in blood were Klebsiella species (8%), followed by Coagulase negative Staphylococci (6.8%), then Enterococci (3.2%). This is in contrast to a previous study done by Deverick et al (2014) whose findings were S.aureus (28%) followed by E.coli (24%) then Coagulase Negative Staphylococci (10%). The observed emergence of K. oxytoca which is an opportunistic pathogen associated with nosocomial infections may be due to poor infection control measures in the newborn unit, overcrowding and also the fact that newborns themselves are susceptible to infections due to immature immune systems. In addition, lack of standard facilities with controlled air flow, entry and exit points and proper sterilization of all gowns and equipment are also contributory. The high rate of growth of Coagulase Negative Staphylococcus may also be due to poor infection control or swabbing practices.

All positive cultures of ascitic fluid were from adult patients from medical wards with none from the surgical wards. This could be attributed to misclassification of ascitic taps under pus aspirates or surgical site infections. The only organism found in this study was P. aeruginosa (0.4%). This was in contrast with a study done by Montravers et al (2009) to identify microbiological profiles of intra-abdominal infections found E.coli to be the highest (27%), followed by Anaerobes (23%) and Streptococci(12%) .

Streptococcus pneumoniae is the most frequently isolated organism from pleural fluids in study conducted at Qatar by Khan et al., unlike to in our study most detected bacteria were Pseudomonas spp. (20%, n=2/10). The reason for this is not clear but may be due to the new vaccine being given as part of routine child immunization activities, which might be have reduced the occurrence of invasive diseases due to Streptococcus pneumoniae or the fastidious nature of bacteria, whereas high prevalence of pseudomonas spp. detected in this study may be indicates the poor way of cleaning and sterility procedure. In our study Pseudomonas spp. was isolated which is in concordance to the study done by Foster et al (2007) and Schultz et al (2004).

**Antibiotic Susceptibility Patterns**

Klebsiella 20(8%) were found predominant isolates among gram-negative bacteria followed by Pseudomonas spp. 15 (6%) and E. coli. 3 (1.2%).

Coagulase negative Staphylococcus 17 (6.8%) were found predominant isolates among gram-positive bacteria followed by Enterococcus 8 (3.2%) and CoPS 4 (1.6%).

Over all the isolated bacteria's 66(98%) had shown Multiple-drug-resistance (MDR ≥ 2 drugs). Among these isolated bacterial gram positive and gram negative had shown multiple drug resistance of 100% (n=36/36) and 96% (n=28/29) respectively.

Among gram positive bacteria all were found to be sensitive to vancomycin except Enterococcus (2). These findings are contrary to a study done in neonates by Qu et al (2010) whereby most coagulase-negative staphylococcal isolates were resistant to penicillin G (100%), gentamicin (83.3%) and oxacillin (91.7%) and susceptible to vancomycin (100%). A different study in Benin by Sina et al (2013) looking at 136 isolates of S.aureus strains from furuncles, pyomyositis, abscesses, Buruli ulcers, and osteomyelitis, from hospital admissions and out-patients found all strains to be resistant to benzyl penicillin, while 25% were resistant to methicillin, and all showed sensitivity to vancomycin. Further local studies are necessary to establish staphylococcal susceptibility patterns to vancomycin to ascertain whether it can still be a drug of choice considering the upward trend of antimicrobial resistance.

The Enterobacteriaceae showed sensitivity against cefepime, amikacin and and resistance against ampicillin and cefoperazone. Against ceftazidime they had 50% sensitivity and 50% resistance. These findings are consistent with a study by Kebira et al (2012) where all isolates of E.coli (100%) were susceptible to Ticarcillin, Piperacillin/Tazobactam, Amikacin, Ofloxacin; and 80% of the isolates were susceptible to Gentamycin, Norfloxacin, and Ceftazidime.

Pseudomonas isolates showed sensitivity towards ceftazidime and amikacin and there is an ampicillin & piperacillin while 73% resistance was found against cefepime.

One study by Reuken et al (2012) showed 63% resistance to ampicillin and 13% to vancomycin and a different study has shown resistance of vancomycin to be on the increase with a rate of ~60% among E. faecium isolates (Wisplinghoff et al 2004). However in

this study Enterococci, most of which were isolated from blood, were found to be sensitive towards Amoxycylav acid but majorly towards vancomycin.

The findings of study: Out of 250 sample taken 69(18.75%) were found to be growth positive.

Overall, Gram positive bacteria (55%) were more common than Gram negative organism (42%). Incidence of blood culture positive cases were more in males (60.8%) than in females (39.2%). Age of patients ranged from newborn to 82 yrs. and most common age group affected was 21-40 years. Klebsiella spp. (28.98%) were the most common isolate followed by CoNS (24.64%), Pseudomonas aeruginosa (21.74%), Enterococcus (11.59%), CoPS (5.78%), Escherichia coli (4.35%). BYC species were isolated in 2.9% of cases. Imipenem (93%) was found to be most effective drug against the Klebsiella species followed by amikacin (90%). While cefoperazone, ampicillin, ceftriaxone were least effective drugs against Klebsiella species. Imipenem (87%) was also the most effective drug against the Pseudomonas species. Moderately effective drugs were ciprofloxacin (73%), amikacin (60%) and piperacillin (47%). Least effective were ampicillin (13%) and ceftriaxone (13%). It was seen that vancomycin and linezolid were the most effective drugs for Gram positive organisms. Least sensitive drugs were cotrimoxazole, erythromycin and ampicillin. Penicillin showed 100% resistance.

The present study provided information on prevalence and antimicrobial susceptibility pattern among common pathogens causing sterile body fluid infections. Among gram negative organism Klebsiella species were the most common isolates & among gram positive most common isolates was CoNS. The antibiotics susceptibility testing showed that imipenem was the most effective drug for Gram negative isolates including Pseudomonas. Among gram positive isolates vancomycin & linezolid were most effective.

Further studies need to be done especially concerning novel antibiotics to establish the best drugs that can be used to tackle these bacteria.

**Short Comings:** The bacterial isolates could not be identified upto species level due to non- availability of biochemical/ identification test to extended period.

**Recommendations:** Identify the profile of bacteria from body fluid with their antimicrobial susceptibility is recommended to clinician for treating infected body fluid. A regular monitoring of antimicrobial resistance patterns of infected body fluid is essential to prevent further emergency and the spread of antimicrobial resistance. Rational use of antibiotics is advised to help curb this trend of increasing antibiotic resistance. Infection prevention and control interventions in the hospital such as promotion of proper personal hygiene and controlled environment e.g. in newborn units is necessary to help control nosocomial infections.

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#### AUTHORS

**First Author** – Dr. Neelam Kholiya, B.D.S., M.Sc. (Medicine) Microbiology, email: dariya\_jaipur@yahoo.com

**Second Author**– Dr. B. L. Dariya, M.B.B.S., M.D., SMS Medical College, Ajmer, email: dariya\_jaipur@yahoo.com

**Third Author** – Dr. Vijaylatha Rastogi, M.B.B.S., M.D., email: [drvjlata@gmail.com](mailto:drvjlata@gmail.com)

**Fourth Author** – Dr. Geeta Parihar, M.B.B.S., M.D., email: drgeetaparihar@gmail.com

**Correspondence Author** – Dr. B. L. Dariya, M.B.B.S., M.D., SMS Medical College, Ajmer, email: [dariya\\_jaipur@yahoo.com](mailto:dariya_jaipur@yahoo.com),  
Mob:+919587004199