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Prevalence and distribution of bacteria isolated from patients with urinary tract infection

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Abstract
The aim of the present study was to isolate and identify bacterial species causes urinary tract infection at M.P. Birla Hospital, Satna, MP A total of 100 patients of all age group clinically diagnosed as catheter associated Urinary Tract Infection (CUTI) were studied to isolate bacteria from urine. Out of 100 CUTI 22 (22%) were culture positive. Among the 20 cases of Group A containing pus cell ≤5/ HPF in the centrifuged deposit of urine, 07 were culture positive, among the 12 cases of Group B containing pus cell 6-10/ HPF, 09were culture positive and among 10 cases of Group C containing pus cell >10/ HPF, all 8 were culture positive. Female is more prone (63.63%) than male (36.36%) for the above infection. it is found that Diabetic (38.4%) was identified as risk factor for maximum patients fallowed by surgical (21.7%). The most common bacterial species isolated were *Escherichia coli, klebsiella, pseudomonas,* and *Staphylococcus aureus* form different diseased patient.

Keywords: urinary tract infection, prevalence, cauti, diabetic, surgical, cardiac

1. Introduction
Health care-associated infections have long been recognized as crucial factors bothering the quality and outcomes of health care delivery. "An infection is considered nosocomial if it becomes evident 48 hours or more after hospital admission or within 30 days of discharge following inpatient care" (Bello *et al*., 2001) [1]. Healthcare-associated infections (HAIs) or nosocomial infections were not present or incubating at the time of admission, comprise a significant burden of illness (Mukerji, Narciso, Moore, *et al*., 2013) [9] HAIs are cause of a major and increasing morbidity and mortality in around the World as well as US. The mortality rates of Healthcare-associated infections (HAALIs) are varying from 5% to 35% that making HAIs is among the ten top leading cause of death. Nosocomial infection is an identified public health problem world-wide with a prevalence rate of 3.0-20.7% and an incidence rate of 5-10 %) (Flodgren *et al.* 2013; Samuel *et al*., 2010) [6, 10]. All admissions 5 %to 10% percent are complicated by HAI in both the US and Western Europe. Annually, In the US alone 1.7 million infections resulting in approximately 99,000 deaths occur (Klevens *et al*. 2007) [8]. More than 177 000 potentially infections (HAIs) occur annually in Australia with sizable attributable mortality (Ferguson *et al*., 2007) [5]. The World Health Organization (WHO) estimates an average of 9 million individuals are affected by nosocomial infections and approximately 1 million patients die each year because of these diseases (Ferguson *et al.*, 2007) [5]. Developing countries were reported to have up to 20 times the risk of contracting a nosocomial infection compared with developed countries (World Health Organization *et al.*, 2008) [15].

2. Material and Method
2.1 Place and period
The study was carried out in the department of Microbiology, M.P. Birla Hospital, Satna, MP, during the period from Jan. 2015 to April 2015.

2.2 Population
A total of 100 clinically suspected of catheter associated urinary tract infection (CUTI) cases were enrolled for this study.

2.3 Selection criteria
The patients who fulfilled the inclusion criteria were enrolled in this study irrespective of age and sex.

2.3.1 Inclusion criteria for community acquired UTI
Clinically suspected catheter associated Urinary tract infection cases were selected on the basis of following cardinal signs/symptoms (CDC criteria) (Savas *et al*, 2006).

1. Urgency
2. Frequency
3. Dysuria
4. Suprapubic tenderness
5. Fever (>100.4^{0}C)
6. Pyuria

2.3.2 Exclusion criteria for community acquired UTI
Febrile causes other than UTI.

2.4 Data collection and recording
All relevant history, clinical findings and laboratory records of every subject was systemically recorded in a pre-designed data sheet. (APPENDIX I)

2.5 Criteria of the different personal parameters used for each study case
2.5.1 Age: Age of each study case was recorded in years.

2.6 Personal hygiene
2.6.1 Collection of urine specimen
The clean catch mid-stream technique was employed to collect urine samples (Ferguson, 2007) [5]. Following the verbal consent of the patient /attendants, a urine sample was collected INA sterile container.

a. For female patients-After proper positioning of thigh, patient will be instructed to spread the labia with one hand and cleanse the area with soaped swabs with the other hand, then pass a small amount of urine into toilet, and finally urinate into the wide mouthed container (Howes and Henry, 2008) [7].
b. For male patient-After washing his hands, clean catch mid-stream urine will be collected with foreskin separated (Collee *et al*, 1996) [4].
c. For catheterized patient-Urine was collected through the draining portal of the urinary catheter using aseptic precaution (Tullu, 1998) [13].

2.6.2 Transport of urine specimen
Approximately 20 ml of urine was collected aseptically in a sterile wide mouthed container. Each sample in the container was properly labeled with patients name, ID number etc. The specimens were then transferred to the laboratory as quickly as possible, usually within 1 hour after collection (Collee *et al*, 1996) [4].

2.7 Examination of urine specimen
2.7.1 Microscopic examination
2.7.1.1 Wet film preparation for centrifuged urine
Five ml of urine samples were poured into a clean and dry 15 ml centrifuge tubes and centrifuged at 3000 RPM for 5 minutes. The supernatant fluid was discarded and one drop of sediment was transferred to a clean labeled glass slide, covered with a clean cover slip and then examined under a light microscope using 10X and 40X magnifications. On the basis of findings of pus cells/ HPF, urine samples were categorized into 3 groups. Group A included all those urine samples having a pus cell count equal or less than 5/ HPF. Group B included pus cell counts ranging between 6 to 10 /HPF and group C were pus cell counts above 10/ HPF (Chowdhury, 1998) [3].

2.7.1.2 Wet film preparation for un-centrifuged urine
One drop of un-centrifuged urine sample was also examined. In un-centrifuged urine pus cell >1 per 7 HPF is significant. The finding of 1 leucocyte per 7 high power fields corresponds with 104 leucocyte per ml (Collee *et al*, 1996) [4].

2.8.2 Cultural examination
Measured amount of urine specimen from each urine sample was inoculated separately into following media:

2.8.2.1 Blood agar: For the isolation of the fastidious microorganisms and to study the type of haemolysis produced by the organisms.

2.8.2.2 MacConkey agar: For isolation of Gram negative enteric bacteria and to differentiate lactose fermenting organisms from non-lactose fermenting organisms.

2.8.2.3 Clad agar: It is a non-selecting differential plating medium for growth and enumeration of urinary tract microorganism. Here indicator dye, bromothymol blue is used to differentiate lactose fermenting from non-lactose fermenting bacteria. Electrolyte deficient prevents swarming of *Proteus.*

2.8.2.4 Muller Hinton agar was used for drug sensitivity

2.8.2.5 Nutrient agar for preservation of organisms. (Collee *et al*, 1996) [4].

2.9 Culture procedure
Sterile urine samples were shaken well in their sterile containers for even distribution of organisms. A calibrated wire loop with internal diameter 3.26mm that hold 0.004 ml of urine were inoculated into the above media. The inoculums were spread with the wire loop on the media plate. They were incubated aerobically at 37°C for 24 hours (Collee *et al*, 1996) [4].

2.10 Reading of the culture plate (Stamm, 2005) [12]
After completion of incubation, the inoculated culture plates will be observed for presence of any bacterial growth. If growth occurs, colony count will be done to calculate the number of colony forming unit per ml of urine.

Interpretation
$\geq 10^5$ CFU bacteria / ml in asymptomatic patients on two consecutive specimens
$\geq 10^3$ CFU bacteria / ml in symptomatic male
$\geq 10^5$ CFU bacteria / ml in symptomatic female
$\geq 10^2$ CFU bacteria / ml in a catheterized patients
Any growth of bacteria on Suprapubic catheterization in symptomatic patients
All significantly bacteriuria cases are termed as “culture positive”, the rest are termed as “culture negative”.

2.11 Identification of the organism (Collee *et al*, 1996; Cheesbrough 2000) [4, 2].

All isolates were subjected to gram staining for initial identification of organism according to their gram reaction, colony morphology and finally by biochemical test. Gram negative bacteria were identified by motility test, indole production, citrate utilization test, urease production and reaction in TSI media. Gram positive bacteria were identified by catalase test, coagulase test and novobiocin sensitivity test.

2.11.1 Escherichia coli

Gram negative bacilli

Colony morphology

MacConkey agar: Produce smooth pink colony

CLED agar: Smooth, circular, 1.5mm diameter, yellow opaque colony

Blood agar: Rounded colonies of 1. 4 mm diameter with or without haemolysis.

Biochemical test

On TSI agar: Yellow slope & butt with gas production

Indole positive, motile and non-producer of urease in MIU medium

Citrate. Negative, Oxidase-Negative

2.11.2 Klebsiella spp

Gram negative bacilli

Colony morphology

MacConkey: Mucoids pink colonies

CLED: Mucoid yellow colonies

Blood agar: Large grey white mucoid colonies

Biochemical test

On TSI agar: Yellow slope & butt with gas production

Indole negative, non-motile and slow urease producer in MIU medium Citrate. Positive, Oxidase-Negative

2.11.3 Proteus spp

Gram negative motile bacilli

Colony morphology

MacConkey agar: Produce non-lactose fermenting colony

CLED agar: Produce non-lactose fermenting colony

Blood agar: Fishy odor, swarming growth

Candida albicans

MacConkey agar

CLED agar: Tiny to small whitish colonies on a blue medium.

Blood agar: Blood agar: moist, opaque colonies are

Biochemical test

On TSI agar: Red slope & yellow butt with gas and H2S production.

Indole negative, motile and urease producer in MIU medium Citrate. Positive, Oxidase-Negative.

2.11.4 Pseudomonas aeruginosa

Gram negative motile bacilli occur as single bacteria, in pairs and occasionally in short chain.

Colony morphology

MacConkey agar: Produce non-lactose fermenting pale colony

CLED agar: Produce non-lactose fermenting green colony

Blood agar: Large flat, spreading colonies, often hemolytic, greenish blue colour pigment production, some produce mucoid colony.

Culture produced characterized grapelike smell of amino acetophenone

Biochemical test

Oxidase- Positive

TSI- Red slope & butt without production of gas & H2S

Motility test: Motile, Citrate. Positive

2.11.5 Enterobacter spp

Gram negative motile bacilli

Colony morphology

MacConkey: Pink (lactose fermenting) colonies

CLED: Yellow colonies Blood agar: Large grey colonies but not so mucoid

Biochemical test

On TSI agar: Yellow slope & butt, produce gas but no H2S.

Indole negative, motile and urease producer in MIU medium

Citrate. Positive, Oxidase-negative

2.11.6 Citrobacter spp

Gram negative motile bacilli

Colony morphology

MacConkey: Pink (late lactose fermenter) colony

CLED: Yellow colony

Blood agar: Grey white colonies

Biochemical test

On TSI agar: Yellow or red slope & yellow butt, produce gas but no H2S.

Indole negative, motile and different strains give different results in urease production in MIU medium Citrate. Positive, Oxidase-Negative

2.11.7 Morganella spp

Gram negative motile bacilli

Colony morphology

MacConkey agar: Small, non-lactose fermenting pale colour, moist colonies

Blood agar: Produce individual colonies.

Nutrient agar: 1-3 mm whitish smooth, colony

Biochemical test

TSI agar: Red slope & yellow butt, few strains produce gas but no H_2S.

Indole- Positive, motile and urease producer in MIU medium Citrate. Positive, Oxidase-Negative

2.11.8 Serratia spp

Gram negative bacilli

Colony morphology

MacConkey- Non lactose fermenter

CLED: Pale colony

Blood agar: White colony

Biochemical reaction
TSI agar: Red or yellow slope & yellow butt, few strains produce gas but no H2S.
Indole- negative, motile and different strains give different results in urease production Citrate. Positive, Oxidase-Negative

2.11.9 Staphylococcus aureus
Gram positive cocci appeared in grape like clusters with some single or paired spherical arrangement.

Colony morphology
MacConkey: Pink color colony, 0.1 to 0.5 in diameter
Blood agar: 1-2 mm in diameter, yellow to cream or white colony. Some strains are beta haemolytic and produces pin head colony.
Nutrient agar: 1 - 3 mm in diameter, convex with shining surface and showing golden
Yellow pigmentation

Biochemical test
Coagulase positive, Catalase positive

2.11.10 Staphylococcus saprophyticus
Gram positive cocci

Colony morphology
Blood agar: Yellow to cream or white. Some strains are beta haemolytic, 1-2 mm in diameter.
Nutrient agar: 1 to 3 mm in diameter, smooth glistening surface Mac Conkey: Growth may not occur on it.

Biochemical test
Coagulase negative, Catalase positive and Novobiocin resistant

2.11.11 Enterococcus spp
Gram positive cocci, occurring in pairs or short chains.

Colony morphology
Blood agar: Mainly non hemolytic, some are alpha or beta hemolytic.
MacConkey: Small dark red magenta colnies.
CLED: Small yellow colonies.

Biochemical test
Catalase - Negative
Bile aesculin- Positive
Able to grow in the presence of 6.5% NaCl.

3. Results

A total of 100 patients of all age group clinically diagnosed as catheter associated Urinary Tract Infection (CUTI) were studied to isolate bacteria from urine. Out of 100 CUTI 22 (22%) were culture positive. (Table 1)
Table 2 shows the relationship between pyuria and culture positivity. Among the 20 cases of Group A containing pus cell ≤5/ HPF in the centrifuged deposit of urine, 07 were culture positive, among the 12 cases of Group B containing pus cell 6-10/ HPF, 09were culture positive and among 10 cases of Group C containing pus cell >10/ HPF, all 8 were culture positive. Culture positivity was directly proportional to pus cell and there is highly significant association of positive culture and pus cell (÷2 value109.78, P> 0.001).

Table 1: Distribution of culture by UTI

Type of UTI	Urine cultured	Culture positive cases	Percentage
CUTI	100	22	22 %

Table III XV ariance in Age and sex distribution of the urine culture samples of CUTI.
Among the samples analyzed and data obtained by the patients of CAUTI it could be assessed that female is more prone (63.63%) than male (36.36%) for the above infection

Table 2: Distribution of infected patient Male and Female

Total no. of male CAUTI patient	Total No. Of CAUTI female
8(36.36%)	14 (63.63%)

Table IV: Age and Sex distribution of the urine culture sampal CAUTI

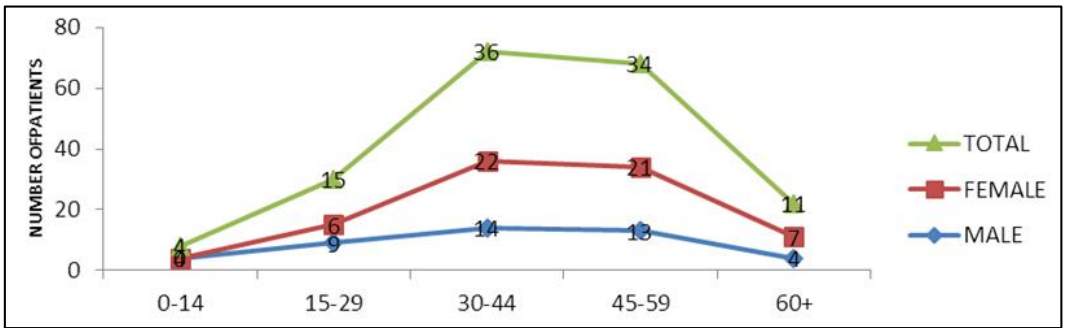

Fig 1: Age of patients

Table V: Prevalence of risk factor among suspected CAUTI patient
From table V it is found that diabetic (38.4%) was identified as risk factor for maximum patients. Surgical (21.7%) was the next to it. The most common organisms isolated were *Escherichia coli, klebsiella, pseudomonas, and Staphylococcus aureus* form different diseased patient.

Table 3: Ariance in Age and sex distribution of the urine culture samples of CUTI

Risk factor Cauti patient	Ward							No. of Infected Patient	Percent
	ICU	POP	PVT	MW	SW	HDU	Total No. of patient		
Diabetic patient	3	No	2	2	1	5	13	5	38.4%
Surgical	No	7	No	No	16	No	23	5	21.7%
Cardiac	5	No	No	No	No	3	8	1	12.5%
Other	1	5	4	13	17	16	56	10	17.8%

Table 4: Microscopic and Macroscopic examination of urine

Pus cell	Centrifuge Urine	Uncentrifuge Urine
Group A (Pus cell count ≤5 / HPF)	20(7 positive)	9
Group B (Pus cell count 6-10 / HPF)	12(9 positive)	2
Group C (Pus cell count >10 / HPF)	10 (8 positive)	Not Seen
Epithelial cell		
Group A (Epithelial cell count ≤5 / HPF)	12	7
Group B (Epithelial cell count 6-10 / HPF)	3	2
Group C (Epithelial cell count >10 / HPF)	2	Not Seen
RBC’		
Group A (Rbc**:** / HPF ≤5 / HPF)	10	2

Table 5: Prevalence of risk factor among suspected CAUTI patient

Albumin	Centrifuge Urine
Group A(Trace)	4
Group B(1-2 +)	11
Group C(3-4 +)	Nil
Urine Sugar (Random)	
Group A(Trace)	1
Group B(1-2 +)	3
Group C(3-4 +)	Nil

4. Conclusion

CA-UTI is an important device-associated health care acquired infection. The use of an indwelling urethral catheter is associated with an increased frequency of symptomatic urinary tract infection and bacteremia, and additional morbidity from non-infectious complications. Infection control programs must develop, implement, and monitor policies and practices to minimize infections associated with use of these devices. A major focus of these programs should be to limit the use of indwelling urethral catheters, and to remove catheters promptly when no longer required. Ultimately, however, the avoidance of CA-ASB will likely require development of biofilm resistant catheter materials.

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