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 followed 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, cautii, 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) [3]. 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°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 systematically 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) [3]. Following the verbal consent of the patient/attendants, a urine sample was collected in a sterile container.

a. For female patients-After proper positioning of thigh, the 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 the 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].

2.7.2 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
≥10^5 CFU bacteria / ml in asymptomatic patients on two consecutive specimens
≥10^4 CFU bacteria / ml in symptomatic male
≥10^5 CFU bacteria / ml in symptomatic female
≥10^6 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”.

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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 pale colony
CLED agar: Produce non-lactose fermenting colony
Blood agar: Fishy odor, swarming growth

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

2.11.5 Enterobacter spp
Gram negative motile bacilli

2.11.6 Citrobacter spp
Gram negative motile bacilli

2.11.7 Morganella spp
Gram negative motile bacilli

2.11.8 Serratia spp
Gram negative bacilli

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 acetonophene

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 H2S.
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.

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

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-14</td>
<td>8(36.36%)</td>
<td>14 (63.63%)</td>
</tr>
<tr>
<td>15-29</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>30-44</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>45-59</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>60+</td>
<td>5</td>
<td>7</td>
</tr>
</tbody>
</table>

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: Arianse in Age and sex distribution of the urine culture samples of CUTI

<table>
<thead>
<tr>
<th>Risk factor Cauti patient</th>
<th>Ward</th>
<th>No. of Infected Patient</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICU</td>
<td>POP</td>
<td>PVT</td>
</tr>
<tr>
<td>Diabetic patient</td>
<td>3</td>
<td>No</td>
<td>2</td>
</tr>
<tr>
<td>Surgical</td>
<td>No</td>
<td>7</td>
<td>No</td>
</tr>
<tr>
<td>Cardiac</td>
<td>5</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 4: Microscopic and Macroscopic examination of urine

<table>
<thead>
<tr>
<th>Pus cell</th>
<th>Centrifuge Urine</th>
<th>Uncentrifuge Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (Pus cell count ≤5 / HPF)</td>
<td>20 (7 positive)</td>
<td>9</td>
</tr>
<tr>
<td>Group B (Pus cell count 6-10 / HPF)</td>
<td>12 (9 positive)</td>
<td>2</td>
</tr>
<tr>
<td>Group C (Pus cell count &gt;10 / HPF)</td>
<td>10 (8 positive)</td>
<td>Not Seen</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Albumin</th>
<th>Centrifuge Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (Trace)</td>
<td>4</td>
</tr>
<tr>
<td>Group B (1-2 +)</td>
<td>11</td>
</tr>
<tr>
<td>Group C (3-4 +)</td>
<td>Nil</td>
</tr>
<tr>
<td>Urine Sugar (Random)</td>
<td></td>
</tr>
<tr>
<td>Group A (Trace)</td>
<td>1</td>
</tr>
<tr>
<td>Group B (1-2 +)</td>
<td>3</td>
</tr>
<tr>
<td>Group C (3-4 +)</td>
<td>Nil</td>
</tr>
</tbody>
</table>

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.

5. References


