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Isolation and Characetrisation of Pathogens from Various Clinical Samples: A Step towards Prevention of Infectious Diseases

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ABSTRACT:

When bacterial diagnosis is considered with its antibiotic profiles, it can do wonders in preventing antibiotic resistance. This of course will help clinicians to write accurate, right and complete prescription without need of broad spectrum or antibiotic combination therapy. This is the only policy that is very simple and that very effective with no big limitations to reduce antibiotic resistance along with Multi drug resistance. Used for disease prevention and infection control procedures this data can be used to protect people now and in future. A total number of 908 clinical samples i.e., urine, blood, pus, other body fluids were collected in the laboratory in the period of two years. The samples were streaked on various culture media for bacterial growth and incubated at 37°C for 24 hours. The plates of the agar media were observed after 24 hours of incubation to identify the types and number of colonies. The plates that were showing no growth were reincubated for next 24 hrs before confirming them as sterile. The results were variable with various types of pathogens in different clinical samples. E.*coli* was found to be the main causative agent in almost clinical infections.

KEY WORDS: antibiotic susceptibility, antimicrobial resistance surveillance, isolation, clinical samples

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INTRODUCTION:

Isolation and characterization of clinical pathogens are the two basic units of surveillance. [1] Isolation and characterization of bacteria from clinical samples have significant role in diagnosing the disease. And knowing the causative agents and flora of that particular region, if come to knowledge, its planning procedure of their prevention may be initiated.

Isolation and characterization techniques in clinical bacteriology have always been slow processes.[2] still, these methods are considered to be gold standard in diagnosis of any infection.[3] New molecular techniques , novel culture techniques have tried to sweep the basic methods of microbiology, but isolation methods remain same for many cases., i.e. culture methods.[4,5]

Although a set of advanced microbiological methods have been developed to fasten the diagnosis, but the methods that Pasteur and Koch described for isolation of bacteria are still being used. [2, 6]

METHODS AND MATERIALS:

The work was undertaken to isolate the causative bacterial agents from various clinical samples.

Data from patients of Nawanshahr, Punjab (rural and urban) for last two years (from

February 2013- February 2015) period was collected from laboratory. Thus the study describes the isolation, identification and surveillance in clinical samples. The study was conducted in a private laboratory of Ludhiana, Punjab. Blood, urine, stool pus and body fluids samples were included in the study.

All biochemical tests like IMViC, oxidase, catalsae, coagulase, urease etc. were used to characterise the isolates. [2, 7]

SAMPLE COLLECTION:

A total number of 908 clinical samples i.e., urine, blood, pus, other body fluids were collected in the laboratory in the period of two years. Out of 908 samples 780 samples were collected from adult females, 106 from adult males, 10 from male children and 12 from female children. All types of clinical samples that were prescribed by clinician, depending on infection in patient, were collected in laboratory aseptically and prior to the use of antibiotics. [8, 9]

PROCESSING:

The samples were streaked on Blood agar, Nutrient agar and Mac-conkey agar and incubated at 37°C for 24 hours. [10, 11] The plates of the agar media were observed after 24 hours of incubation to identify the types and number of colonies.[12] The plates that were showing no growth were reincubated for next 24 hrs before confirming them as sterile.[13,14]

All blood samples were taken in Brain heart infusion broth medium and then these samples were subcultured on Maconkey agar plates, Nutrient agar plates, and blood agar plates, thrice on alternate days. [15, 16]

All types of colonies on the plates were further isolated from each other. The isolated colonies were next processed for identification by various tests. [8, 9]

IDENTIFICATION OF ISOLATES:

Isolated pure colonies were identified by colony morphology characteristics, gram staining and biochemical tests. Depending upon their colony morphology i.e., small, pin point, tiny or large, etc. and their gram staining characters, they were separated as Gram Positive cocci or Gram negative bacilli.[8,9] Initially a total of 185 isolates were taken, which were screened primarily by colony characteristics, gram staining and furoxin disc test. Isolates that found to be Aerobic spore bearing bacilli and micrococcus by these three parameters, were ruled out being non pathogenic. [17, 28]

Furthermore, Different biochemical test were used to differentiate between gram positive and gram negative bacteria.[30] Gram positive cocci were differentiated by catalase and coagulase tests in laboratory.[27] The GPC that gave both catalase and coagulase positive results, was considered to be Staphylococcus aureus. Depending upon other catalase and coagulase reactions along with their haemolytic activity on blood agar, they were again identified as Enterococcus spp. [18, 19]

On the other hand, for GNB's, colour of colonies on maconkey agar plate differentiated them into lactose fermenters (pink coloured colonies) or lactose non fermenters (pale colonies). Furthermore, for lactose fermenter GNBs their Indole test was performed with Citrate test, motility test, Urease test, Triple sugar Iron agar test and oxidase tests were performed. [20, 21]Indole test was exempted in case of Non lactose fermenters.[22] Depending upon each biochemical reaction results, isolates were identified as Escherichia *coli*, Klebsiella *spp.*, Acinatobacter *spp.*, Pseudomonas *spp.*, Citerobacter *spp.*, Enterobacter *spp.*, Enterococcus *spp.*, Staphylococcus *aureus* etc.[2,7](figure 1.)

Figure 1. Various biochemical reactions to identify the isolates. (From right to left-Indole, citrate, motility, urease, TSI)

RESULTS:

Results obtained from various clinical samples indicated that microbes varied in different samples. There was no

uniformity in microbes in different samples. Maximum no. of samples obtained were urine sample (83.9%). whereas, minority no. of samples received were body fluids (0.7%). However, the positivity rate of both types of samples was 8.2% and 42.8%. These results show that urine infections are more prevalent in the particular region, but the microbial isolation techniques may not be sufficient in diagnosing UTI's. Number of other types of culture like pus and blood culture was 13.3% and 3.6% respectively and the positivity rate of both cultures was vice versa, 9% and 38% respectively.

Since urine samples received were very much higher in number than other samples, we must know if any specific causative organism responsible for UTI in specific region. These isolates of urine samples must be kept in vigil to control UTI's as per their mode of transmission, their quantization on culture plate antibiotic susceptibility pattern, etc. the results of urine culture showed that the majority of isolate was Escherichia *coli* (2.8%) in contrast with Enterobacter *spp.* That was only 0.2% in all urine culture positive isolates. Other Gram negative bacteria isolated were Klebsiella *spp.*, E.*coli* and Enterobacter *spp.* 6.3%, 34.9% and 3.1% respectively. Non fermenter bacteria, Pseudomonas *spp.* and Acinatobacter *spp.* remained in equal proportion in UTI's i.e., 7.9 %.(Table 1.)

Out of Gram positive bacteria isolated in urine cultures, Enterococcus *spp.* was predominant (2.3%). Whereas, other Gram positive cocci like Staphylococcus *aureus* contributed just 0.9% to UTI's.

For patients with Urinary tract infections, age and sex wise distribution of patients showed the highest no of female adult patients (73.8%). On the other hand, female child patients were the fewest patients who reported UT infections. Out of total samples only 9.7% and 1.1% male adult and male child patients reported UTI. In female patients E.*coli* was the most predominant pathogen responsible for UTI. Whereas, in adult males Enterococcus *spp.* and Pseudomonas *spp.* were equally responsible for causing UTI.

Importantly, Pseudomonas *spp.* Were the most common pathogen that was isolated from both male and female children patients in clinical samples like blood, urine and pus.

In pus cultures, the most affected category of patients was adult males (47.3%). Female adult patients (39.4%)

also showed not a less number of infections. Male children (5.2%) and female children (7.8%) reported occasionally for pus cultures. (figure2.) In both sexes, Enterococcus *spp.* were the most common isolate found in pus cultures almost same like of urine culture, where after E.*coli*, Enterococcus *spp.* has found to be second important pathogen causing UTI.

Enterobacter *spp.* and Pseudomonas *spp.* are least responsible organisms (1%) for causing pus infections. Data of pus culture showed variety of organisms (Acinatobacter *spp.*, Citerobacter *spp.* and Proteus *spp.*; 3%) equally causing infection of skin and wound. As far as lactose fermenters are concerned causing skin, wound or pus infections, the data revealed Klebsiella *spp.* (2%) caused half of the infections caused by E.*coli*(4%) in pus.

Proteus *spp.* showed a significant role in pus infections in male patients only (7.8%) with not even a single case of Proteus infection in females, same as Citerobacter *spp.* and Klebsiella *spp.* here in this case, although both organisms have been reported very few in numbers(5.2% and 2.6% respectively), but both pathogens has infected male adult patients only.

As blood culture samples received were very less, their positivity rate is 9.0%. No male patient has reported any infection in blood. Only two organisms Staphylococcus *aureus* (6.0%) and pseudomonas *spp.* (3.0%) in females and Male children patients have been reported in data of the study. Both of the organisms may signify hospital acquired infections, as both organisms are considered to be nosocomians. This data itself has queried the concerned hospital personnel or physicians that why and how the nosocomian infections arise in blood cultures.
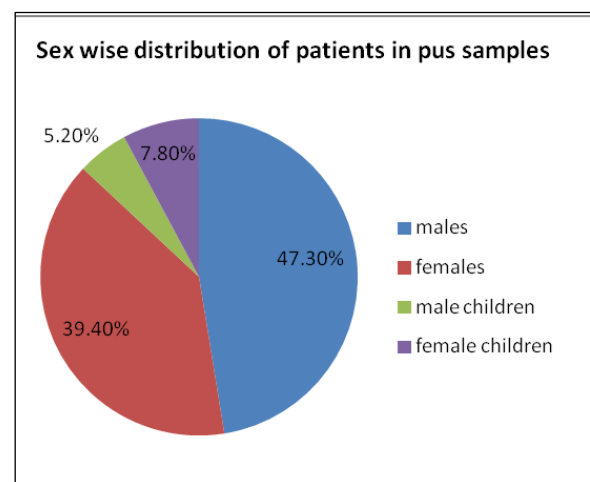

Figure 2. Sex wise distribution of patients in pus samples

Table 1. Different types if Isolates in each clinical sample.

	urine(total no. 762)	blood(total no.33)	stool(total no.6)	pus(total no.100)	other body fluids(total no.7)
Staphylococcus *aureus*	7	2	0	7	1
Enterococcus *spp*.	18	0	0	14	2
Klebsiella *spp*.	4	0	0	2	0
Pseudomonas *spp*.	5	1	0	1	0
Acinatobacter *spp.*	5	0	0	3	0
Proteus *spp.*	0	0	0	3	0
Citerobacter *spp.*	0	0	0	3	0
E.*coli*	22	0	0	4	0
Enterobacter *spp.*	2	0	0	1	0
Salmonella *typhimyruium*	0	0	4	0	0
TOTAL	63	3	4	38	3

DISCUSSION:

The influence and impact of pathogens on particular society has been documented in various studies but in Punjab, not too many studies on microbial surveillance have been done. The research mainly yields the point that in common infections, there is geographical and climatic difference that creates difference in species of pathogens in similar samples. Our study showed that in this region of Punjab though urinary tract infections are most common the positivity rate is very less, leading to the suggestion that the diagnosis and treatment should be strengthened to rule out true urine infections.[22] In case positivity rate of body fluids, it is very much exposed to sight that the positivity rate is higher due to severe or chronic illness. lesser %age of fluid culture may not signify that people in particular geography are less afflicted with fluid infections, but it may be considered that due to inaccessible sample collection sites for body fluid in patients, the samples of body fluids cannot be collected frequently. Hence a single number of pathogen may be considered as critical for selection of antibiotics severe illness.

The difference in spp. distribution in each sample in this study is striking. As noted, the frequency of the bacteria is likely to be influenced by many predisposing / underlying factors. A study documented by Ramsamy et al., in trauma patients, Klebsiella spp. were isolated maximum (25%) in contrast with E.coli (19%), where as in our study we report more E.coli (34.9%) than Klebsiella spp. (6.3%).[23] According to Ramsamy's study Acinatobacter spp. were considered to be colonizers unless they showed a role in severe sepsis.[23] In contrast with our study, acinatobacter in was considered as sole organism in pus and urine infections. Given this observation, differences in society, living style, and geography, age and sex are responsible for difference in species in particular samples all over world.[23] The study shows that the main victim of UTI is adult females in contrast with female children and male patients. This may be due to human anatomy that why females catch UTI more often. Moreover, in South Africa traumatic patients showed that there were more than 15 species of gram negative bacilli isolated; indicating that sudden distressed immunity may encounter rare and vast number of bacteria. Other supporting factors of encountering with rare pathogens may be patient's hospital admission, nosocomian infections or iatrogenic infections. [24] No fungal infection was detected in this study in contrast with Ramsamy's study. [23]

In comparing these data it is first and foremost to understand clearly that the results of most surveillance studies have thinkable biases.[27] In this the contemplations should be made about the population surveyed, geography surveyed or methods used for surveillance. Significant differences may exist in microbial patterns and these differences may likely to affect data to compare different studies. [26] Thus similar methods,

population and sites may enhance the affectivity of surveillance and help in real eradication multi drug resistance and common bacterial infections due to unawareness of common man. [26] Ultimately, this study has provided the basic trend of pathogens in particular region in specific infections. That will help clinicians to rule out some super bugs from easily handled bacteria causing infections with precluding any broad spectrum antibiotics.

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