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ISOLATION OF ESBL PRODUCING *PROTEUS* FROM UTI PATIENTS

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Abstract: A total of 45 consecutive *Proteus* recovered during the study period in 100 urine sample of UTI patients. 10 isolates were ESBL producer and 35 isolates were non-ESBL producers. A susceptibility disk containing Piperacillin/Tazobactam was placed as the inhibitor of β -lactamase in the center of the plate. Enhancement of zone of inhibition of disc of Piperacillin alone towards the disc containing Piperacillin/Tazobactam, showing a figure of eight impression were considered as ESBL producer. All recovered isolates were resistant against ampicillin, amoxicillin, ceftazidime, ceftriaxone, tetracycline, chloramphenicol, gentamycin, cefotaxime and sensitive against imipenem, amikacin, and ciprofloxacin and meropenem. The prevalence of extended spectrum β -lactamase producing *Proteus* in urine sample of UTI patients was 10%. Majority of Gram negative bacteria showed susceptibility towards Amikacin, Nitrofurantoin, and Gentamicin. The present studies the incidence of urinary tract infection was high in the age group of 20-29. The drugs norfloxacin and ciprofloxacin, the relatively fluoroquinolones were found to be the most effective against Uropathogenic isolates. Aqueous extract of *Hibiscus Rosa-sinensis* has shown highest range of antibacterial activity in terms of inhibition zone diameter (23mm) in comparison to *Catharanthus rosea* (20), *Tagetes* (20), *Dolichos Biflorus* (17) in urine samples against *Proteus sp*. From the present study it has been concluded that aqueous extracts of *Hibiscus Rosa sinensis* possess a significant amount of antimicrobial agents and is most effective against Urinary tract infection.

Keywords: ESBL, Chloramphenicol, Gentamycin and β -lactamase, *Proteus*.



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INTRODUCTION

UTI defined as the microbial invasion of any of the tissues of the urinary tract extending from the renal cortex to the urethral meatus. The prevalent organisms that are usually isolated from UTIs patients are *E. coli*, *Staphylococcus aureus*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Proteus* spp. *Streptococcus faecalis* and *Enterobacter* spp). Gram-negative bacteria have been found most frequently in UTIs. The *Enterobacteriaceae*, were the most frequent pathogens detected, causing 84.3% of the UTIs¹. *Escherichia coli* causes about 85% of community-acquired UTIs². *Proteus mirabilis* is a common cause of UTI in individuals with long-term urinary catheters in place or individuals with complicated urinary tracts. Urinary tract infection is a common health problem in all over the world. In males, the prevalence of UTI is about 0.3%, but increases (13–40%) in the older age group (≥ 65 years) because of prostatic diseases and urologic manipulations³. The aim of this study was to characterize ESBL-producing gram negative bacteria isolated from the urine of patients based on their susceptibility to antimicrobial agents.

MATERIALS AND METHODS

Sample Collection:

Urine of (UTI) patients is used as sample for the isolation of *Proteus* which is collected from Civil Hospital, Paonta Sahib. Isolation of *Proteus* sp. is done by selective plating on Phenylalanine Agar and incubated at 37°C for 24 hrs.

Isolation and Identification of Bacterial strains:

Proteus sp. was characterized by Colony Characteristics, Gram Staining, and Biochemical identification. Screening of ESBL producing *Proteus* is determined by Double Disc Diffusion test.

Antibiotic Sensitivity Test:

Antibiogram Profiling of ESBL producing *Proteus* in UTI patients is determined by Kirby-Bauer Disc Diffusion method⁴.

Collection of Plant material:

The plant material used was fresh flowers collected directly from the herbal garden and flower sellers of Paonta Sahib. The flowers were allowed to air dry in shade and then homogenized into fine powder for extraction process.

Extraction of plant material

Aqueous extraction:

10g of each flower air dried powder was weighed and soaked separately in 100ml distilled water in a conical flask and filtered next day using sterile filter paper into a sterile conical flask and subjected to water bath evaporation, where the aqueous solvent was evaporated at its boiling temperature 100°C.

Antimicrobial activity of plant extract

Preparation of inoculums:

Isolated colonies are inoculated in Mueller Hinton Broth medium. Incubated at 37°C for 4-6 hrs.

Medium:

Mueller Hinton Agar medium used was for test organism.

Antibacterial assay:

In Muller Hinton agar plates wells were cut and swabbed with different cultures and wells were then filled with 50µl of aqueous extracts of flowers and seeds separately and the plates were kept for incubation at 37°C for 24 hrs. After 24 hrs, the plates were observed for the zone of inhibition of growth and the zones were measured.

Results and discussion

A total of 45 consecutive *Proteus* recovered during the study period in 100 urine sample of UTI patients. 11 isolates were ESBL producer and 35 isolates were non-ESBL producers. All recovered isolates were resistant against ampicillin (25mcg), amoxicillin (25 mcg), ceftazidime (30 mcg), ceftriaxone (30 mcg), tetracycline (30 mcg), chloramphenicol (30 mcg), gentamycin (25 mcg), cefotaxime (30 mcg) and sensitive against imipenem (30 mcg), Amikacin (30 mcg), and ciprofloxacin (25 mcg) and meropenem (10 mcg). The ESBLs prevalence rate in the present study was 10% much lower than those reported from Nigeria (16.7%) Obiogbolu et al (2009), Egypt (75%) Khaled Z (2009). Urinary tract infection (UTI) is a serious health problem affecting millions of people each year. Gram negative bacilli are most frequent uropathogens and are resistant to commonly used antibiotics. Among the aqueous extracts of four different flowers i.e. Hibiscus Rosa sinensis, Catharanthus rosea, Tagetes, Dolichos Biflorus used for the determination of antibacterial activity of recovered isolates causing UTI. Aqueous extracts of Hibiscus Rosa sinensis has shown maximum activity in terms of zone of inhibition in comparison to Catharanthus rosea, Tagetes, and Dolichos Biflorus. Zone diameter (*mm) in urine samples

against *Proteus* species were Hibiscus Rosa sinensis (23), Catharanthus rosea (20), Tagetus (20), Dolichos Biflorus (17). Figure 5 and 6 show antimicrobial activity of isolates by using different flower extracts: 1(Hibiscus rosa-sinensis-23), 2(DolichosBiflorus-17), 3(Catharanthusroseus-19), 4(Tagetus-20). In this study, different microorganisms were used to screen the possible antimicrobial activities of *Hibiscus Rosa sinensis* extracts. The extract and fractions of Carica papaya were tested for antibacterial activity against clinical isolates of *Proteus mirabilis*. The water extracts are more effective. The large zone sizes produced by the plant extract against the test bacteria, especially the aqueous extracts is an indication of the potency of the bioactive components of the plant against all the test bacteria. From the present study it has been concluded that aqueous extracts of Hibiscus Rosa sinensis possess a significant amount of antimicrobial agents.

Figure 1 shows control, Fig 2, 3, shows Antibacterial activity of recovered isolates by using different antibiotics (ESBL screening)

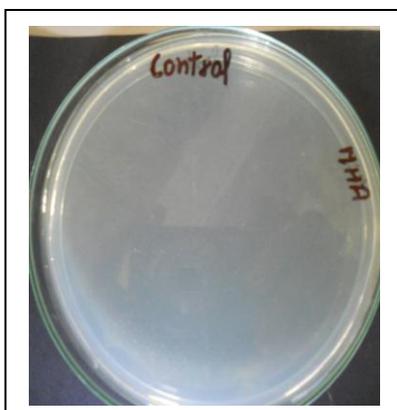


Figure.1. (Control)



Figure.2. Antibacterial activity of recovered isolates

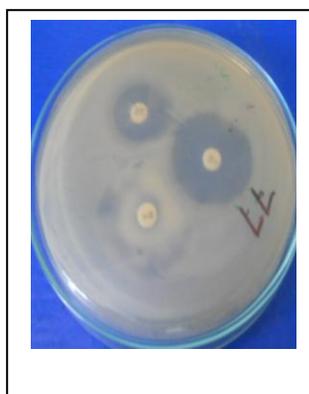


Figure 3 (*Proteus* ESBL)

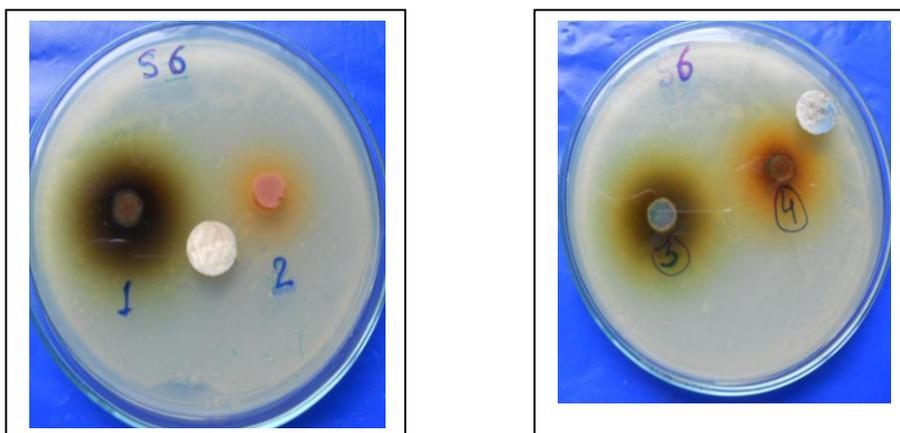


Fig 5&6: Antimicrobial activity of isolates by using different flower extracts: 1(Hibiscus rosa-sinensis-23), 2(DolichosBiflorus-17), 3(Catharanthusroseus-19), 4(Tagetus-20).

CONCLUSION

Gram-negative bacteria were the major cause of urinary tract infection. The prevalence of extended spectrum β -lactamase producing *Proteus* in urine sample of UTI patients was 10%. Majority of Gram negative bacteria showed susceptibility towards Amikacin, Nitrofurantoin, and Gentamicin. In conclusion, our data indicate the spread of ESBL type in clinically gram negative isolates in General hospitals. Nearly one-third of urine samples collected showed significant bacterial growth with *E. coli* as predominant isolate followed by *Proteus sp.*, *Klebsiella sp.* and others Present findings together with previous ones are suggestive of need of periodic monitoring of antibiotic sensitivity pattern of the bacterial isolates to provide effective treatment and thereby to make it more cost effective particularly in the impoverished countries like elsewhere and ours. In conclusion, the present studies the incidence of urinary tract infection was high in the age group of 20-29. The drugs norfloxacin and ciprofloxacin, the relatively flouroquinolones were found to be the most effective against Uropathogenic isolates followed by Gentamycin and nalidixic acid. The aqueous flower extracts of four different plants were used for the determination of antibacterial activity of *Proteus sp.* causing UTI. Aqueous extract of Hibiscus Rosa-sinensis has shown highest range of antibacterial activity in terms of inhibition zone diameter (23mm) in comparison to Catharanthus rosea (20), Tagetus (20), Dolichos Biflorus (17) in urine samples against *Proteus sp.* These extracts were very effective against recovered isolates. From the present study it has been concluded that aqueous extracts of Hibiscus Rosa sinensis possess a significant amount of antimicrobial agents and is most effective against Urinary tract infection. In future we can use these herbal extract for prepration of herbal tablet and syrup as best alternate"

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