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European Journal of Experimental Biology, 2015, 5(4):45-49



Study of virulence determinants and antimicrobial activity of drugs against urinary tract infection pathogens

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ABSTRACT

The uropathogens possess a wide array of virulence factors which help them in pathogenesis. The present study investigated the virulence factors produced by uropathogens and also determine the antimicrobial activity of drugs against the pathogens. E. coli was documented to be the most prevalent uropathogen. 60% of uropathogens were found to be siderophore producers. 73% isolates were found to be protease producers. A total of 74% uropathogens were rhamnolipid producers. Isolates were found to be maximally sensitive towards streptomycin and oxacillin.

Key words: Uropathogens, virulence factors, siderophore producers, protease producers, rhamnolipid producers.

INTRODUCTION

Urinary tract infections typically occur with the entry of bacteria into the urinary tract through the urethra and subsequent multiplication in the bladder. It may be manifested as a variety of clinical conditions like asymptomatic presence of bacteria in the urine to severe infection of the kidney with resultant sepsis. It is reported to be one of the most common diseases encountered affecting people of all ages. *Escherichia coli, Klebsiella* sp., *Proteus mirabilis, Pseudomonas aeruginosa, Acinetobacter* and *Serratia* are the causative bacteria of most of the urinary tract infections. *E. coli* is the main causative agent of UTI [1]. The wide spectrum of virulence factors enables the uropathogens to establish the infection. Virulence of uropathogens has been attributed to cellassociated factors like alginate, lipopolysaccharide (LPS), flagellum, pilus and non-pilus adhesins as well as with exoenzymes or secretory virulence factors like protease, elastase, phopholipase, pyocyanin, exotoxin A, exoenzyme S, hemolysins (rhamnolipids) and siderophores [2, 3, 4]. The present study investigated the virulence factors of uropathogens and also the antimicrobial effect of drugs on the uropathogens.

MATERIALS AND METHODS

2.1 Microbial culture

A total of 100 uropathogens were taken from culture collection center, department of microbiology, Dolphin (PG) Institute of Biomedical Sciences, Dehradun, India.

2.2 Chracterization of isolates

The morphological and biochemical characterization of recovered uropathogens was carried out. Cell morphology (Gram's reaction, cell shape and arrangement) of isolates were studied. The various biochemical tests viz., Oxidase test, Indole-Methyl Red-Voges-Proskauer-Citrate Utilization test (IMViC), Triple Sugar Iron (TSI) test, Urease test and Nitrate reduction tests were carried out according to [5].

2.3 Characterization of virulence factors

The various virulence factors viz., Siderophore, Rhamnolipid and Protease were studied qualitatively. All incubations were done at $37+1^{\circ}$ C.

2.3.1 Siderophore production

Siderophore production was assayed according to [6]. Isolates were spot inoculated on Chromeazurol "S" agar. Isolates exhibiting an orange halo zone after 48-72 h of incubation were considered as positive. Their zone diameter was measured.

2.3.2 Protease production

The bacterial isolates were spot inoculated on gelatin agar. The isolates exhibiting opaque zone around growth after treatment with 1% Tannic acid were considered as positive and their zone of hydrolysis was measured.

2.3.3 Rhamnolipid production

It was estimated according to [7]. All isolates were inoculated on Rhamnolipid production medium. Isolates exhibiting blue colour were considered as positive.

2.4 Antibiotic sensitivity assay

All isolated were tested for antibiotic sensitivity by Kirby-Bauer disc diffusion method [8] on Mueller-Hinton agar. Cultures were inoculated by swabbing with standard inoculum over the entire agar surface. The agar surface was allowed to dry for 3-5 minutes before applying the antibiotic discs using sterile forcep. Four Antibiotic discs were placed equidistantly on 90 mm Petriplate. The Plates were incubated aerobically at 37+ 1°C in incubator for 16 to 18 hrs. Next day the zone of inhibition was measured. Antibiotics used were: AC- Amoxycillin (30mcg); AK-Amikacin (30mcg); CO- Cotrimaxazole (1.25/23.75mcg); CS- Cefoperazone/Sulbactam (50/50mcg); CE-Cefotaxime (30 mcg); CF- Cefoperazone (75mcg)

RESULTS

3.1 Prevalence of uropathogens

The distribution of different pathogens is depicted in Figure 1. E. coli was the dominant isolate (49%).



Fig.1: Distribution of uropathogens

3.2 Virulence characterisation of uropathogens

Uropathogens exhibited various virulence characteristics *viz.*, siderophore production, protease production and rhamnolipid production (Fig. 2 & 3).

3.2.1 Siderophore production

60% of uropathogens were found to be siderophore producers. Amongst them 20% were weak producers exhibiting 1-6 mm zone. 3% isolates were medium producers exhibiting 7-15 mm zone while 6% isolates were strong producers giving zone 16-53mm.

3.2.2 Protease production

73% isolates were found to be protease producers. 54% isolates were weak producers exhibiting 1-4mm zone of hydrolysis while 16% were medium producers exhibiting 5-10mm zone of hydrolysis. 3% isolates were strong producers giving 11-19mm zone of hysrolysis.

3.2.3 Rhamnolipid production

A total of 74% uropathogens were rhamnolipid producers.



Fig. 2: Distribution of virulence properties (%) amongst uropathogens



Fig. 3: Frequency of Siderophore and Protease Producers amongst uropathogens

3.3 Antibiotic sensitivity testing

Isolates were found to be maximally sensitive towards streptomycin and oxacillin. They were found to be maximally resistant towards trimethoprim and tobramycin (Fig.4).



Fig. 4: Antimicrobial susceptibility of antibiotics against recovered UTI pathogens

CIP- Ciprofloxacin; TOB- Tobramycin; CLIN- Clindamycin; E- Erythromycin; GEN- Gentamicin; C- Chloramphenicol; AK- Amikacin; PIT-Piperacillin; S- Streptomycin; TR- Trimethoprim; OX- Oxacillin

DISCUSSION

Urinary tract infections (UTIs) are a serious health problem affecting humans throughout their life span. It is the second most common infection of any organ system and the most common urological disease in the United States, with a total annual cost of more than \$3.5 billion [9]. The present study was carried out to characterize the virulence factors produced by uropathogens and to test their antimicrobial susceptibility towards drugs.

In the present study E. coli was the most prevalent uropathogen. It has been very well documented in literature that E. coli is the most prevalent uropathogens in 80-90% of cases [1]. Uropathogenic strains are characterized by the expression of distinctive bacterial properties, products, or structures referred to as virulence factors because they help the organism overcome host defenses and colonize or invade the urinary tract. Virulence factors of recognized importance in the pathogenesis of urinary tract infection (UTI) include adhesins (P fimbriae, certain other mannoseresistant adhesins, and type 1 fimbriae), the aerobactin system, hemolysin, K capsule, and resistance to serum killing [10, 11]. The predominance of Escherichia coli is due to the presence of P fimbriae, due to which it may adhere on specific receptors of uroepithelial cells. The more virulence factors a strain expresses, the more severe an infection it is able to cause. The currently defined virulence factors clearly contribute to the virulence of wild-type strains but are usually insufficient in themselves to transform an avirulent organism into a pathogen, demonstrating that other asyet-undefined virulence properties await discovery [11]. Virulence of P. aeruginosa is multifactorial and has been attributed to cellassociated factors like alginate, lipopolysaccharide (LPS), flagellum, pilus and non-pilus adhesins as well as with exoenzymes or secretory virulence factors like protease, elastase, phopholipase, pyocyanin, exotoxin A, exoenzyme S, hemolysins (rhamnolipids) and siderophores [2, 3, 4, 12]. These factors have been shown to play an important role in pathogenesis of P. aeruginosa induced infections like respiratory tract infections, burn wound infections and keratitis [13]. However, limited reports are available regarding role of these virulence traits in urinary tract infections. In the present study 60% of uropathogens were found to be siderophore producers. Siderophore forms water soluble complexes with Fe³⁺ [6]. They chelate iron and thus enable pathogenic microbes to compete successfully for iron in the highly iron-restricted environment of the host's tissues and body fluids. Protease is considered to be another important virulence property which help in invasion in host tissues. In the present study 73% isolates were found to be protease producers. Rhamnolipids are naturally occurring glycolipids which promote dissemination of cells [7]. In present study a total of 74% uropathogens were rhamnolipid producers. The study of virulence factors of uropathogens would enable to devise treatment strategy. Isolates were found to be maximally sensitive towards streptomycin and oxacillin. They were found to be maximally resistant towards trimethoprim and tobramycin.

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