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A study on prevalence, virulence factors and antibiotic susceptibility of *Klebsiella oxytoca* isolates in a tertiary care centre

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Abstract

Background: *Klebsiella oxytoca* is a Gram-negative member of the human microbiota but can cause urinary infection and other infections. It can be detected in the intestines of about 2 to 10% of healthy subjects, and until recently, *K. oxytoca* was considered to be a commensal member of the enteric microflora. However, it has been shown that *K. oxytoca* is in fact an intestinal pathobiont and the causative agent of antibiotic-associated hemorrhagic colitis (AAHC). The aim of this work was, therefore, to isolate *Klebsiella* strains from all possible source of infection, and to modify and set up a simple technique to detect the serum resistance in these isolates and biofilm formation in these strains by test tube method. We also want to see antibiogram of *K. oxytoca* isolates since this may guide pre-emptive therapy. Objectivesis the isolation and identification of *K. oxytoca* from different samples and performing serum resistance and biofilm assessment by test tube method in these strains as well as observing resistance to common antibiotics in these bacteria. Material and methods of the study sample consisted of one hundred (100) clinical isolates of *K. oxytoca*. Gram staining and standard biochemical tests used for clinical diagnosis, among the isolates. A total of 100 isolates of *Klebsiella oxytoca*, from various samples (stool, urine, pus, sputum, blood). Out of these, 58 infected patients were male and 42 patients were female.

Key Words: *Klebsiella oxytoca*, Serum resistance, Biofilm formation, Antibiogram.

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Introduction

Klebsiella oxytoca is a Gram-negative member of the human microbiota but can cause urinary infection and other infections[1]. It can be detected in the intestines of about 2 to 10% of healthy subjects, and until recently, *K. oxytoca* was considered to be a commensal member of the enteric microflora [1, 2]. However, it has been shown that *K. oxytoca* is in fact an intestinal pathobiont and the causative agent of antibiotic-associated hemorrhagic colitis (AAHC) [2]. It is very important to know which infections are caused by this pathogen and what is the usual pattern of antibiotic resistance in this pathogen in order to guide the clinician in antibiotic therapy.

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Virulence factors of this pathogen are also important to know.*K. oxytoca* is implicated in not only urinary infection but also antibiotic associated colitis [3]. Under conditions of intestinal dysbiosis, a state of microbial imbalance,*K. oxytoca* unleashes its pathogenic potential. Several factors can perturb the intestinal microbiota during the life span of an individual, including immune deficiency, infections, dietary changes, and drugs, like antibiotics [4, 5]. The consequences of antibiotic-induced intestinal dysbiosis range from diarrheal symptoms to intestinal inflammation and infection. The characteristics of AAHC are sudden onset of bloody diarrhea and abdominal cramps during penicillin or cephalosporin therapy. The antibiotic penicillin is considered critical for triggering dysbiosis, as *K. oxytoca* exhibits a natural resistance to penicillins[5]. Rapid colonic overgrowth of *K. oxytoca* follows during the acute phases of AAHC [3]. The pathogenicity of *K. oxytoca* in colitis is not understood, but a correlation

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has been observed between isolates originating from AAHC patients and the secretion of cytotoxin(s) [1, 2, 6]. Besides the potential to induce colitis under certain circumstances, enteric carriage of *K. oxytoca* may be important for the transmission of antibiotic resistance genes to other bacteria and as a source of nosocomial infections [7, 8]. Indeed, this bacterium and the closely related species *Klebsiellapneumoniae* are important human pathogens causing hepatobiliary infections and infections of the urinary tract and soft tissue, in addition to nosocomial pneumonia [9-11]. In recent years, multidrug-resistant strains of both species have emerged as an important problem in the health care system [7, 12]. So far, no typing method has successfully identified a clonal relationship between *K. oxytoca* isolates with respect to the particular infections they cause, their isolation source, or their toxicity [6, 13]. One property that has been suggested to be important in the ability of bacteria to cause infection is resistance to the bactericidal effect of human serum. In the present study, *Klebsiella* strains from different sources will be examined for sensitivity to human serum. Bacteria belonging to the genus *Klebsiella* frequently cause human infections, gastrointestinal tract being the main reservoir of *Klebsiella*[14]. The most common area of bacterial colonization of this pathogen is the urinary tract: in the community setting is reported to cause from 2 to 15% of cystitis cases [15]. Also, the incidence of *Klebsiella pneumoniae* increases in the hospital infections [14]. While different typing methods are useful epidemiological tools for infection control, recent findings about *Klebsiella* virulence factors have provided new insights into the pathogenic strategies of these bacteria [15]. *Klebsiella*produces fimbriae that mediate attachment to host mucosal surface, a capsule that protect against phagocytosis and other immune responses, and similar to all Gram negative organisms immunosuppressive lipopolysaccharide (LPS) [14]. Biofilms are complex communities of microorganisms attached to a surface or interface enclosed in an exopolysaccharide matrix of microbial and host origin to produce a spatially organized three dimensional structure [18, 19]. Biofilms are universal, occurring in aquatic and industrial water systems as well as a large number of environments and medical devices relevant for public health. Using sophisticated analytical tools, biofilm researchers now understand that biofilms are not unstructured, homogeneous deposits of cells and accumulated slime, but complex communities of surface-associated cells enclosed in a polymer matrix containing open water channels. Microorganisms growing in a biofilm are highly resistant to antimicrobial agents by one or more mechanisms.

Biofilm-associated microorganisms have been shown to be associated with several human diseases, and to colonize a wide variety of medical devices [17]. The role of biofilm formation and development by bacteria has been suggested to be an important stage in the pathogenesis of *Klebsiella*[20, 21]. When the formation of biofilm is conceived in the urinary tract, always the catheter related infections (CAI), one of the first recognized and most studied biofilm diseases, must be included. It is clear that biofilms are associated with urinary tract infection (UTI) where indwelling device are not the cause: bacteria show to form biofilm on bladder mucosal tissue and on the mucosal surface of the acini of prostate tissue in a rat model of bacterial prostatitis [16]. The formation of biofilms within the urinary tract is one of the best explanation for the recurrent and chronic infections [16]. The urinary tract is protected from pathogen colonization by the flushing action of sterile urine, the sloughing of uroepithelial cells and a glycosaminoglycan layer. The resulting limitations on the therapeutic options demand new measures for the management of the infections produced by the responsible pathogens. Then, research on alternatives therapies are increasing, that include probiotic products at different tracts or mucosa. More specifically, in the lower urinary tract, organisms as *Lactobacillus species* prevent pathogens from attaching and establishing a tissue infection [22]. The aim of this work was, therefore, to isolate *Klebsiella* strains from all possible source of infection, and to modify and set up a simple technique to detect the serum resistance in these isolates and biofilm formation in these strains by test tube method. We also want to see antibiogram of *K. oxytoca* isolates since this may guide pre-emptive therapy.

Material and methods

The proposed study is a Descriptive (lab-based observational) study was carried out in the Department of Microbiology, All India Institute of Medical Sciences, Patna, from November 2016 to October 2017. The study sample consisted of one hundred (100) clinical isolates of *K. oxytoca*. Gram staining from the mucoid lactose fermenting colonies and standard biochemical tests used for clinical diagnosis, among the isolates, *K. oxytoca* were identified on the basis of their colony morphology, characteristics in Triple sugar iron, Christensen's urea agar and Simmons citrate media and differentiated from *K. pneumoniae* by indole positivity. Which were inoculated on Cysteine lactose electrolyte deficient agar (for urine) and Sheep blood agar and MacConkey agar (other samples) and incubated overnight at 37^{0}C. After isolation and

identification of *K. oxytoca* from different samples. We perform serum resistance and biofilm assessment by test tube method in these strains and observing resistance to common antibiotics in these bacteria. Antibiogram: Isolates will be inoculated on Mueller Hinton agar by Lawn culture and antibiotic susceptibility following standard guidelines (CLSI) (Kirby Bauer method)[23]. The following antibiotics will be tested: Ampicillin (10 mcg.), Amoxyclav (30 mcg.), Cotri-moxazole (25 mcg.), Levofloxacin (5 mcg.) and Azithromycin(15 mcg).Serum resistance testing technique: Isolates will be suspended in 200µl Peptone water up to 1 MacFarlane suspension. This was divided into two halves. To one half 300 µl fresh human serum was added and to the other half 300 µl peptone water was added. The two tubes were incubated for 90 minutes at 37^0C and then from each tube subcultures were made on either half of a nutrient agar plate and incubated overnight at 37^0C. Growth obtained were compared next day and upto 99% reduction in growth obtained from serum is considered as susceptible to serum. Biofilm formation: Growth from culture plates were inoculated in 5ml peptone broth and after overnight incubation at 37°C, next day washout with normal saline one times then add 0.5%

safranin for 1 minute, then wash with normal saline twice after drying the test tube we observe for biofilm formation.Statistical methods are not needed in this study since it is descriptive only.

Results

From November 2016 to October 2017, a total of 100 isolates of *Klebsiella oxytoca*, from various samples (stool, urine, pus, sputum, blood). Out of these, 58 infected patients were male and 42 patients were female. From these 100 samples, serum resistance was seen in 55 isolates, biofilm formation in 35 isolates. Both serum resistance and biofilm formation was present in 30 isolates. With respect to antibiogram, ampicillin was the most resistant (80 isolates) drug, followed by amoxyclav (71 isolates) and cotrimoxazole (36 isolates). Most sensitive among all the antibiotics used was azithromycin (74 isolates) followed by levofloxacin (69 isolates).There is no any side effect. Hopefully some new insights will come up regarding pathogenesis and antibiogram which will help in instituting empirical therapy in infections produced by *K. oxytoca*.
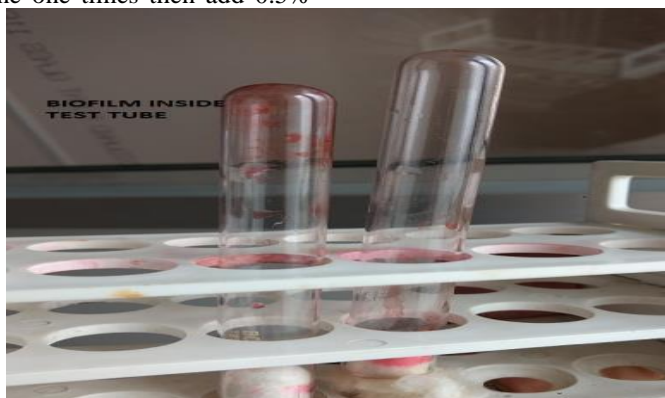
Fig. 1: Test for biofilm forming ability

Discussion

These findings will be important and literature is scarce in this topic, so this study will be interesting to see and carry out. Serum resistance in isolates, especially urinary isolates will help clinicians to know that the particular serum-resistant urinary isolate has propensity to cause septicemia on gaining access to bloodstream from urinary tract. Biofilm forming isolates will indicate that therapy needs to be tailored accordingly.

Conclusion

This study demonstrates the presence of important virulence mechanisms viz. biofilm forming ability, serum resistance in a high proportion of *Klebsiella oxytoca* isolates which may be responsible for its persistence in the environment as well as in the human body. Demonstration of resistance to multiple drugs tested indicates the emergence of multi-drug resistant *Klebsiella oxytoca* isolates in our clinical setting raising a concern of difficulty in treating infections caused by the mentioned organism.

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