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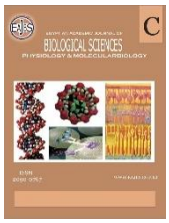

# Isolation, Characterization, and Antibiotic Sensitivity Pattern of Gram-Negative Bacteria Causing Chronic Bacterial Prostatitis.

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**ABSTRACT**

Bacterial prostatitis (BP) is an infection of the prostate gland, it can be acute (ABP) or chronic (CBP). CBP badly affects patient's life quality, and it can cause significant morbidity if not properly treated. In this study, expressed prostate secretion from 10 patients that are diagnosed with chronic bacterial prostatitis at Kasr Al-Ainy hospital, Egypt is used to isolate the common causative bacteria for CBP. Results indicated that Gram negative bacteria were predominant since 54 Gram-ve bacterial isolates were obtained out of the total 62 isolates. Gram-ve bacterial isolates were grouped according to their oxidase test results and to lactose fermentation capability into three groups. The largest group contained 38 isolates of oxidase negative, lactose fermenters and related to *Enterobacter cloacae* complex. The oxidase positive lactose non-fermentors group contained 9 isolates while the oxidase negative lactose non-fermentors group contained 7 isolates. They were identified by 16S rRNA sequence homology to be a strain of *Pseudomonas stutzeri* and *Providencia stuartii* respectively. Antibiotic sensitivity pattern of chosen representative isolates indicated that all of them were sensitive to ciprofloxacin followed by ampicillin while resistance to cefaclor and aztereonam was prevalent. Other tested antibiotics recorded different sensitivity patterns. *Enterobacter cloacae* was the most prevalent.

This study targeted mainly the isolation of the prevalent bacterial pathogens causing chronic bacterial prostatitis in Egypt and to determine their antibiotic resistance pattern for better future treatment.

## INTRODUCTION

Prostatitis has been classified into four categories according to the National Institutes of Health (NIH) that are: acute bacterial prostatitis (ABP), chronic bacterial prostatitis (CBP), nonbacterial prostatitis and prostatodynia (Murphy *et al.,* 2009). ABP (type I) is a severe infection of the prostate gland that is associated, mainly, with Gram-negative bacterial infection (Lipsky *et al.,* 2010). The new United States (US) classification retains the first two types (acute and chronic bacterial prostatitis) but non-bacterial prostatitis and prostatodynia, are classified as inflammatory and non-inflammatory chronic pelvic pain syndrome (Schaeffer, 1999 &Zhang *et al.,* 2020). Acute bacterial prostatitis, usually associated with urinary tract infection (UTI), represents a urological emergency and may require a longer course of treatment than other forms of UTI. The correct choice of an antibiotic for treatment should be based on its ability to penetrate prostatic tissue. Chronic bacterial prostatitis (type II) is a distressing issue for patients since it has a significant negative impact on their life quality and a high likelihood of recurrence (Magri *et al.,* 2007).

Recurrent urinary tract infections and the persistence of a pathogenic bacterium in the prostatic fluid can both be used to reliable diagnosis of CBP, the absence of one of these features excludes the diagnosis. CBP-causing bacteria can be cultivated from expressed prostatic secretions (Pfau, 1986 & Budía *et al.,* 2006). Traditional culture method plays an important key role in the detection of bacteria inhabiting the male reproductive tract (Krieger *et al.,* 2005). Chronic bacterial prostatitis is commonly caused by Gram-negative enterobacteriaceae, occasionally by *Pseudomonas* species, and, rarely, by Gram-positive enterococci. It is currently thought that atypical microorganisms such as *Chlamydia trachomatis*, *Ureaplasma urealyticum*, and *Mycoplasma hominis* could be involved in some cases of CBP; however, under standard conditions, culturing and identifying these species is challenging (Lipsky *et al.,* 2010).

Although the choice of prescribed antibiotics usually is based on antibiotic sensitivity test of the isolated bacteria from patient's EPS, results have often been poor due to inadequate penetration of antibiotic through the prostatic epithelium into the prostatic fluid. Because bacteria in CBP is not completely eradicated, Patients might experience recurrent urinary tract infections. A chronic bacterial prostatitis cure can be said to have been achieved if repeated cultures for urine and prostatic secretion remained sterile for at least six months (Pfau, 1986 & Naber *et al.,* 2000). Despite the acidic nature of prostatic fluid of healthy males (Oelke, 2020), patients with CBP have prostatic secretions with an alkaline rather than an acidic pH. Newer quinolones such as ciprofloxacin are zwitterions which allow concentration in acidic as well as in alkaline prostatic fluid (Dalhoff and Weidner, 1988 & Naber *et al.,* 2000).

The purpose of this study was to isolate and identify the most common causative bacteria in patients diagnosed with CBP in Kasr Al-Ainy hospital, Cairo, Egypt for better understand of their spread in Egyptian hospitals and to identify their antibiotic sensitivity patterns for improved future treatment.

## MATERIALS AND METHODS

### Harvesting of Prostatic Secretions:

Expressed prostatic secretions (EPSs) were used as the study samples, because they are produced from the prostate and while they pass through the urethra, they could acquire bacteria, EPS could reflect the bacterial communities inhabiting the prostate and the urethra. Voiding was performed once to make the bladder empty, followed by application of 75% ethanol solution for urethra and glans sterilization. A physician aseptically collects the prostatic fluid into sterile cups (Xiao *et al.,* 2013).

### Isolation of Bacteria from EPS of Chronic Bacterial Prostatitis Patients:

To explore the pathogenic bacteria in EPS of patients suffering from chronic

prostatitis, a total of 100 µl of prostatic fluid was diluted up to 0.5ml with 0.8% sterile saline solution. Samples from 10 patients were plated on nutrient agar (NA) culture medium (Jorgensen *et al.,* 2015) then, incubated at 30°C for 48 hours. All morphologically different colonies (cultured from each individual patient sample) were streaked on NA to obtain pure cultures. All pure cultures were re-streaked on Mackonkey agar (Eaton, *et al.,* 2005) plates to select the Gram -ve bacterial isolates. Microscopic examination for Gram-stained smears from colonies grown on NA was performed for cell shape determination and also Gram reaction result confirmation. Oxidase test was also performed for Gram negative isolates to differentiate enterics from non-enterics. Based on colony color on Mackonkey agar, cell shape and Oxidase test results, and as described in identification flow charts of Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994), isolates were divided into three groups, Group I, oxidase positive, lactose non-fermentors; Group II, oxidase negative lactose fermenters and group III, oxidase negative lactose non-fermentors. A representative number of isolates for each group (A single isolate from group I and also from group III and 5 isolates from group II) were randomly chosen for genomic and/or biochemical identification as appropriate. A single bacterial isolate of the most common type is needed for the forthcoming work of this study.

**Species Level Identification for Selected Representative Isolates of Group I, II and III:**

**Genomic Identification Through 16SrDNA Data Analysis:**

Genomic DNA extraction and purification, PCR amplification of 16S rDNA, DNA fragments sequencing, and data dnalysis were performed as follow:

***Genomic DNA Extraction and Purification:***

Total genomic DNA was extracted from overnight cultures of chosen isolates that grown aerobically on nutrient broth (NB) at 30°C with shaking at 150 rpm. 1.0 mL of the overnight culture for each isolate was centrifuged at 5000 rpm for 1 min., after the removal of the culture filtrate, the bacterial pellets were washed by resuspending each of them in 1 mL TE buffer (100mM Tris, 10 mM EDTA) and repelleted as above. DNA minipreps were prepared using Promega Wizard® genomic DNA purification kit (New York, USA) according to the manufacturer's instructions. The purified DNA was kept at -20 °C for future use.

***PCR Amplification of 16S rDNA:***

The 16S rRNA gene was amplified from the genomic DNA using the 16S rRNA gene universal primers synthesized by Clontech (Heidelberg, Germany). The nucleotide sequence of the forward primer (27F) is 5`- AGAGTTTGATCMTGGCTCA G-3` and the reverse primer (1492R) is 5`- TACGGYTACCTTGTTACGACTT -3` (Heuer *et al.,* 1997). Amplification was performed in 50 µL reaction volumes as described in Mehanni and Abd El-Aziz, 2019.

***DNA Fragments Sequencing and Data Analysis:***

PCR product of 16S rRNA gene fragments were sequenced by automated ABI-3730xl model sequencer (Applied 132 Biosystems, CA, USA) according to instructions of manufacturer. Sequencing data for five isolates were assembled using Serial Cloner (Version 2.6) program and compared with 16S rRNA gene sequences for bacteria currently available in the GenBank database by BLAST homology search (www.ncbi. nlm.nih.gov/BLAST). Multiple sequence alignments with 16S rRNA gene homologous sequences retrieved from GenBank were performed using ClustalW with default parameters (Higgins *et al.,* 1994); genetic distances were estimated depending on Kimura 2-parameter model (Kimura, 1980); and phylogenetic tree was constructed by Neighbor-Joining method (Saitou & Nei, 1987) using MEGA 6.0 Software (Tamura, 2013).

**Nucleotide Sequence Accession Number:**

The GenBank accession number of the 16S ribosomal RNA gene sequences for five of isolated bacterial strains (of chronic bacterial prostatitis diagnosed patients) were,

MAG-06 (OK087614), MAG-07 (OK087615), MAG-08 (OK087616), MAG-09 (OK087617) and MAG-10 (OK087618).

**Biochemical Identification Using GN24 kit:**

For precise identification on biochemical basis, GN24 kit from diagnostics was used (a standardized identification system for common species of Gram-negative bacteria was used according to the manufacturer's instructions (DIAGNOSTICS Inc, Galanta, Slovak Republic).

**Antibiotic Sensitivity of Isolated EPS Bacterial Strains:**

Antibiotic sensitivity of isolated bacteria was determined by disc diffusion method (Bauer A 1966). Thermo Scientific™ Oxoid™ antimicrobial susceptibility discs were used in this study. The susceptibility towards a particular antibiotic is determined from the zones of bacterial growth inhibition surrounding the antibiotic discs.

## RESULTS

**Isolation of Bacteria from EPS of Chronic Bacterial Prostatitis Patients:**

A total of 62 pure bacterial cultures (isolates) were obtained on nutrient agar plates from all test samples (10). A 54 Gram -ve bacterial isolates were obtained on MacConkey agar out of 62 total isolates number obtained on NA. As indicated from results, the majority of bacteria isolated from EPS were Gram negative bacteria. Gram staining revealed that all Gram -ve bacterial isolates were rod-shaped. Group II (oxidase negative lactose fermentors) recorded the highest number of isolates (38) compared to 9 of group I (oxidase positive, lactose non-fermentors) and 7 of group III (oxidase negative lactose non-fermentors). Isolates within each group had the same colony characteristics on both NA and MacConkey agar plates.

**Species Level Identification for Selected Representative Isolates for Groups I, II and III:**

**16S rRNA Gene-Based Identification of EPS Isolated Bacteria:**

Fragments of amplified 16S rDNA, from EPS bacterial isolates, using 27F and 1492R 16S rDNA universal primers were cloned and sequenced. Sequencing data were reviewed by BLAST homology search for similarity with other prokaryotic taxa. 16S rRNA gene sequence of three isolates out of 5 amplified represent group II (oxidase negative lactose fermenters) had good sequencing data that showed close similarities with records of *Enterobacter cloacae*. Phylogenetic tree (Fig. 1) constructed between the pairwise of isolated strains sequences of 16S rDNA and the closely similar homologs showed that the closest strain to isolate MAG-06 (OK087614) was *Enterobacter cloacae* JX965901.2, while *E. cloacae* MT636553.1 was the closest strain to isolates MAG-07 (OK087615) and MAG-10 (OK087618) with the least distance in between. The other two isolates, their sequencing data were not good enough to process and their identification was restricted to the biochemical method only.

Isolate MAG-08 (OK087616), the representative to group III (oxidase negative lactose non-fermentors), showed close similarities with records of genus *Providencia*. The highest similarity was recorded to *Providencia stuartii* (MH107231.1) as indicated from the constructed Phylogenetic tree (Fig. 2). The representative isolate to group I (oxidase positive lactose non-fermentors), MAG-09 (OK087617) showed close similarities with records of genus *Pseudomonas*. Analysis of the constructed phylogenetic tree indicated that *Pseudomonas stutzeri* (JN 378750.1) strain is the closest (Fig. 3).

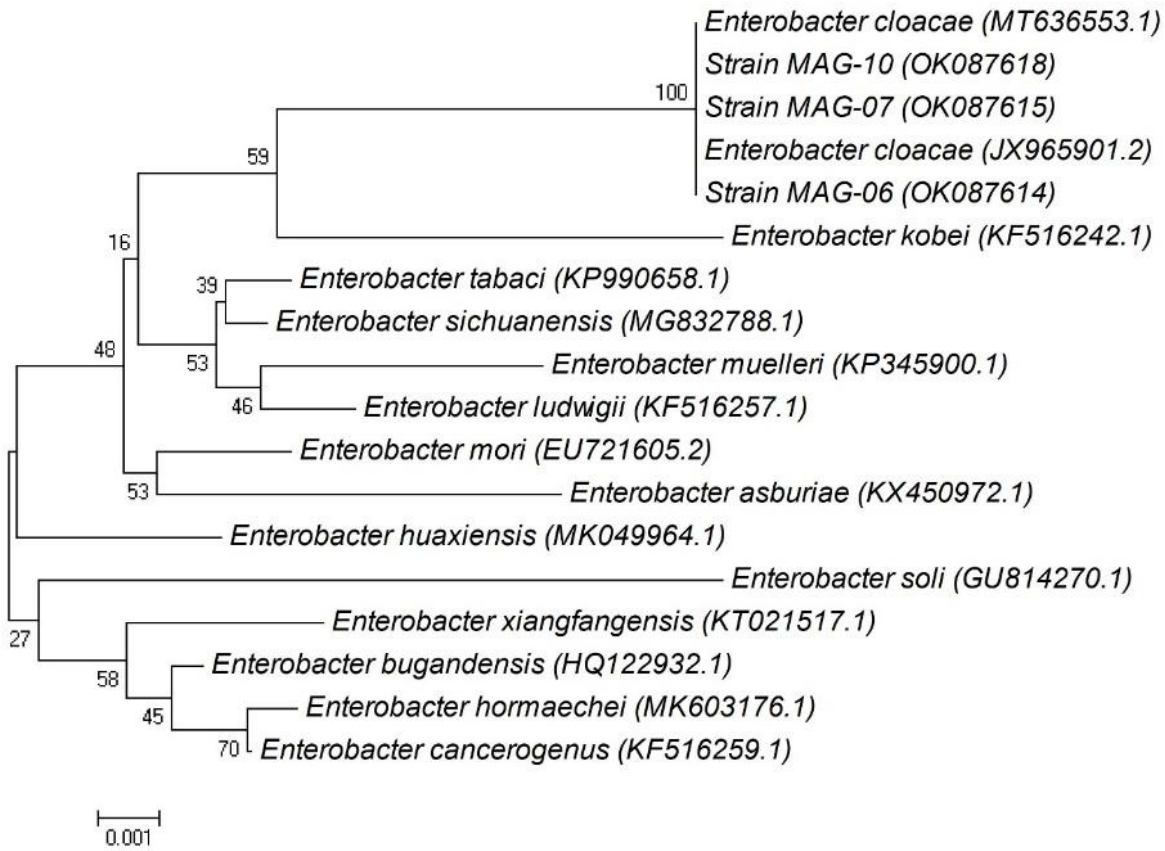


**Fig. 1:** Neighbor-Joining phylogenetic tree constructed for the isolated Enterobacter cloacae strain MAG-06 and its closely related homolog Enterobacter cloacae JX965901.2, also, the isolated E. cloacae strains MAG-07 and MAG-10 and their closely related Enterobacter cloacae MT636553.1 strain. Distances used were computed using the Kimura 2-parameter model. Numbers at nodes refer to bootstrap values for 1000 replicates and scale bar denotes 0.05% nucleotide divergence.

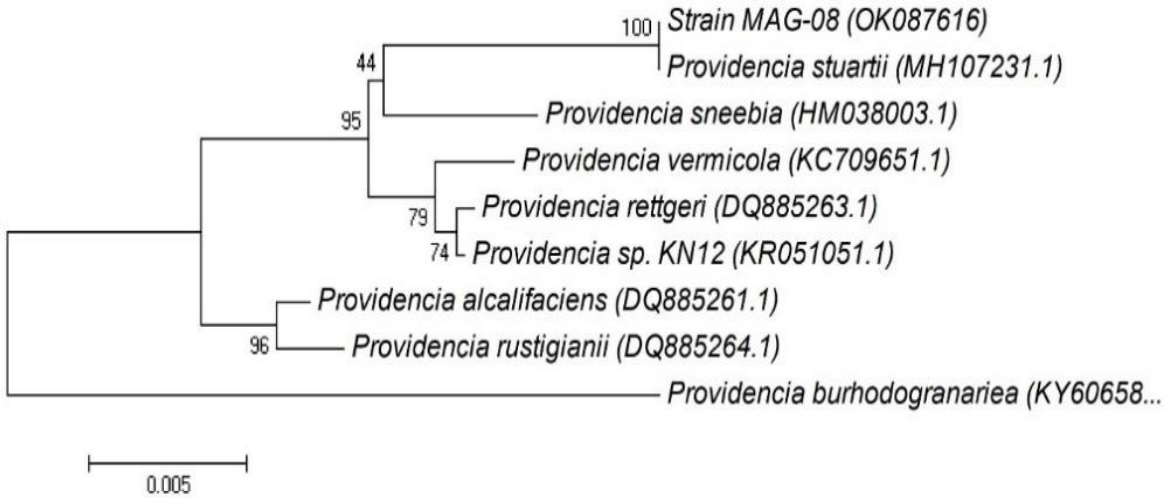


**Fig. 2:** Neighbor-Joining phylogenetic tree constructed for Providencia stuartii strain MAG-8 and the closely related homolog Providencia stuartii (MH107231.1). Distances used were computed using the Kimura 2-parameter model. Numbers at nodes refer to bootstrap values for 1000 replicates and scale bar denotes 0.05% nucleotide divergence.

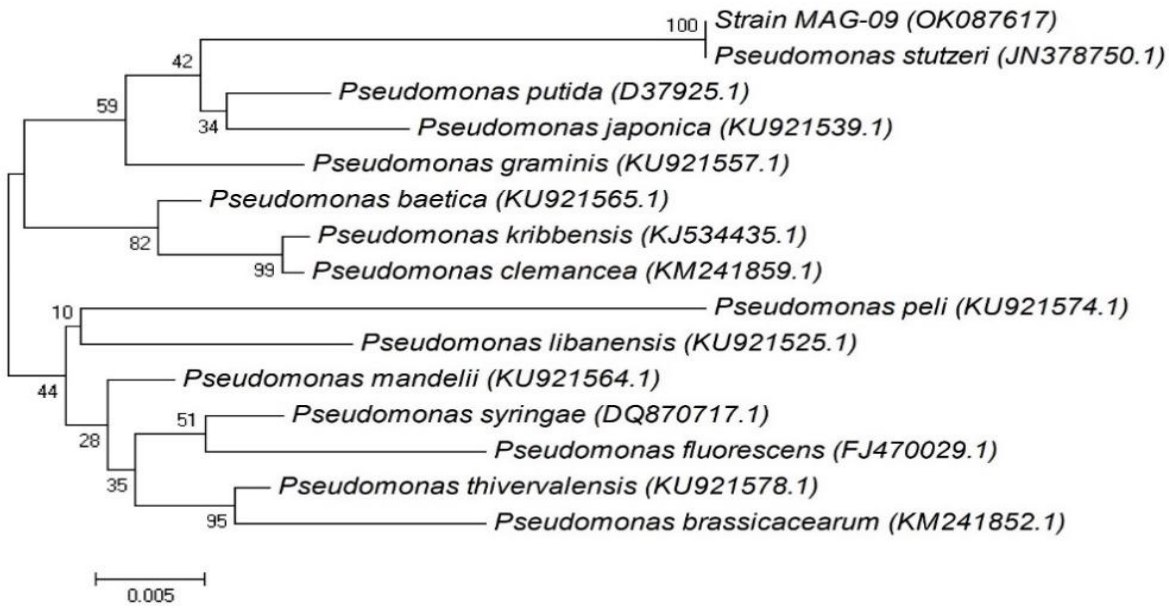


**Fig. 3:** Neighbor-Joining phylogenetic tree constructed for Pseudomonas stutzeri strain MAG-9 and the closely related homolog Pseudomonas stutzeri (JN 378750.1). Distances used were computed using the Kimura 2-parameter model. Numbers at nodes refer to bootstrap values for 1000 replicates and scale bar denotes 0.05% nucleotide divergence.

**Biochemical Identification Using GN24 kit:**

The two isolates of group II designated as Pb1 and Pb2 (that the sequence data of their 16s rDNA were not good enough to follow), were biochemically identified using GN24 kit. Results obtained by the help of evaluation software on the company website, were identified as *Enterobacter kobei* with percent of identification 98.95 (excellent) and 88.87 (very good) respectively.

**Antibiotic Sensitivity of Isolated EPS Bacterial Strains:**

Antibacterial susceptibility of selected EPS bacterial isolates is shown in Table 1. As indicated from results, isolates of *Enterobacter cloacae* complex exhibited variable pattern of sensitivity towards the same antibiotics. Results also showed that all isolates were susceptible to ciprofloxacin to different extents. Results also indicated that all isolates were resistant to the used monobactam antibiotic (aztreonam), except *E. cloacae* strain Mag-07 and *E. kobei* strain Pb2. Resistance pattern towards cefaclor was also prevalent. *E. kobei isolates* Pb1 and Pb2 recorded different antibiotic sensitivity pattern since they were isolated from different patients. *E. kobei* strain Pb1 recorded resistance to 4 of 7 tested antibiotics. *E. cloacae* isolate MAG-06, MAG-07 and MAG-10 (Isolated from different CBP diagnosed patients) also exhibited different antibiotic sensitivity patterns since MAG-06 isolate recorded higher resistance pattern (Table 1). For isolates *Providencia stuartii* MAG-08 and *Pseudomonas stutzeri* MAG-09, both were resistant to 3 of 7 tested antibiotics of which Cefaclor and Aztereonam.

**Table 1:** Antibiotic susceptibility of EPS isolated bacteria from chronic bacterial prostatitis diagnosed patients.

| Family | Antibiotic | Isolated EPS bacterial strains | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *Enterobacter cloacae* complex | | | | | Others | |
| | | MAG-06 | MAG-07 | MAG-10 | Pb1 | Pb2 | MAG-08 | MAG-09 |
| Beta-lactam (aminopenicillin) | Ampicillin | 2.5 | 4.1 | 2.5 | -ve | 2 | 2.3 | 2.9 |
| Aminoglycosides | Gentamycin | 1.9 | -ve | 2.4 | 2.1 | 2.2 | -ve | 2.2 |
| beta-lactam (monobactam) | Aztreonam | -ve | 2.1 | -ve | -ve | 1.7 | -ve | -ve |
| fluoroquinolones | Ciprofluxacin | 4.5 | 4.1 | 3.5 | 2.9 | 2.6 | 2.3 | 2.9 |
| Macrolide | Azithromycin | -ve | 3 | 2 | -ve | 3 | 0.9 | -ve |
| Polyketide | Rifampicin | 0.9 | 4.1 | 1.7 | 0.7 | 1.6 | 3.1 | 0.7 |
| Cephalosporins | Cefaclor | -ve | 2 | 2.4 | -ve | 1.5 | -ve | -ve |

## DISCUSSION

Chronic bacterial prostatitis (CBP) is one of the important factors affecting male reproductive health that is characterized by a high prevalence, low cure rate, frequent recurrence, and severely affect patient's quality of life. An increased risk of benign prostatic hyperplasia and prostate cancer have found to be correlated with chronic prostatitis (Cheng *et al.,* 2007). Expressed prostatic secretions (EPSs) were used as the study samples because they are produced from the prostate and could acquire bacteria while they pass through the urethra, so they reflect the composition of bacterial communities inhabiting both prostate and the urethra. The 16S rRNA gene positive rate in CBP/CPPS is similar to that among patients with localized prostate cancer (Keay *et al.,* 1999& jiang *et al.,* 2013), so future studies should be performed to investigate the role of bacterial community agents causing CBP/ CPPS in prostate cancer development. Identifying the causative bacterium is important for antibiotic selection and the determination of a treatment strategy in the management of any infective disease such as chronic prostatitis, in general, clinical features and treatment methods vary according to the causative microorganism.

In this study, we found that the most prevalent bacterial pathogens from EPS of 10 patients diagnosed with CBP belonged to genus *Enterobacter*. Species level identification of isolated *Enterobacter* strains indicated their relation to species of *Enterobacter cloacae* complex (ECC), three of them (MAG-06, MAG-07 and MAG-010) are related to *E. cloacae* while Pb1 and Pb2 are related to *E. kobei*. The remaining two isolates were related to *Providencia stuartii* (MAG-8) and *pseudomonas stutzeri* (MAG-09).

The *Enterobacter cloacae* complex (ECC) includes common nosocomial pathogens (*E. cloacae* is the most important member within this taxon) that can cause a wide variety of infections such as pneumonia, urinary tract infections, and septicemia. *Enterobacter cloacae* complex includes seven species that are *Enterobacter asburiae*, *Enterobacter carcinogenus*, *Enterobacter cloacae*, *Enterobacter hormaechei*, *Enterobacter kobei*, *Enterobacter nimipressuralis*, and *Enterobacter mori*. All these species are genotypically very close, with more than 60% DNA-DNA homology (Hoffmann and Roggenkamp, 2003 & Annavajhala *et al.,* 2019). Our results clearly indicated that the genotypic relatedness between the three isolated bacterial strains (MAG-06, MAG-07 and MAG-010), that their genomic identification using 16S rDNA sequence homology indicated their grouping with *Enterobacter cloacae*, and the closest species in the assembled Neighbor-Joining phylogenetic tree, *Enterobacter kobei,* is 59% (Fig. 1). This reflects the close genomic relatedness between the three isolated *E. cloacae* strains to the other isolated two *Enterobacter* strains Pb1 and Pb2

(biochemically identified as *E. kobei*).

Most clinical laboratories in developed countries use commercially available kits or semiautomated systems for routine phenotypic identification of *Enterobacter* spp. that are limited to *E. cloacae* and *E. asburiae*. A case study of a nosocomial urosepsis caused by *E. kobei* that was previously identified as *E. cloacae* (Tan *et al.,* 1977& Gandbold, *et al.,* 2023). Biochemical tests or molecular methods such as 16S rRNA, rpoB and hsp60 gene sequencing should be applied for a more accurate identification and discrimination of the other species in this genus (Mezzatesta *et al.,* 2012). The accurate identification of species and subspecies remains a challenge. The phylogeny of the genus, particularly that of the *E. cloacae* complex, was significantly improved after the rapid modification of genome sequencing (Liu *et al.,* 2013).

*Enterobacter* species are members of the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), which are described as the leading cause of resistant nosocomial infections (Canton *et al.,* 2002). *Escherichia coli* and *Enterobacter cloacae* complex are very common human enterobacteriaceae pathogens. A generalized strategy of infection control is quite difficult to apply for chronically colonized patients because these bacteria intestine resident and can acquire resistance determinants (specially with uncontrolled use of antibiotics) and so becoming Multiple drug resistant (Lucet *et al.,* 1999& Aira *et al.,* 2019).

In our study, results indicated that resistance to the monobactam, Aztreonam, was prevalent in the isolated bacteria from EPS (5 of the 6 enterobacteriaceae strains) and for the non-enteric *P. stutzeri* MAG -09. *Enterobacter kobei* (was previously in a group included in *E. cloacae*) have been isolated from various clinical samples: urine, blood, sputum (Kosako *et al.,* 1996). Strains of *E. kobei* differ from those of *E. cloacae* by a negative Voges–Proskauer (VP) test. However, a new VP-positive strains causing urinary tract infection has been characterized (Hoffmann, Schmoldt, *et al.* 2005). Concern about the species is rising because they can produce extended-spectrum beta-lactamases (ESBLs) that break down some of commonly used antibiotics, including penicillins and cephalosporins rendering them ineffective in treating infections (Zhou *et al.,* 2017).

*Providencia stuartii* is an opportunistic, biofilm-forming pathogen from the *Enterobacteriacae* family. *Providencia* species isolated from catheter-associated urinary tract infections usually exhibit multiple resistance to antibiotics, which contributes to the high mortality of patients with *Providencia* bacteremia. *P. stuartii* is generally the second most common species isolated from blocked indwelling catheters after *proteus mirabilis* (Armbruster *et al.,* 2017). As shown from Table 1, the isolated *Providencia stuartii* MAG-08 was resistant to cephaclor, aztreonam, gentamycin and exhibited low sensitivity to azithromycin. Enterobacteriaceae are the predominant pathogens in acute and chronic bacterial prostatitis (Weidner *et al.,* 1991& Magri *et al.,* 2020).

Although *Pseudmonas. stutzeri* is widely distributed in the environment and rarely causes infections, opportunistic pathogen strains have been clinically isolated. *P. stutzeri* infection have been reported in association with other infections such as bacteremia/septicemia and many other ailments including UTI (Alwazzeh *et al.,* 2020) and reported to be more sensitive antibiotics than its most closely related species, *P. aeruginosa* (Noble and Overman, 1994). Interestingly, with the exception of fluoroquinolones, resistant *P. stutzer* strains have been isolated for almost all antibiotic families (Lalucat *et al.,* 2006). In current study, *P. stutzeri* strain MAG-09 recorded resistance against some tested antibiotics such as cephaclor, azithromycin, aztreonam and showed low sensitivity to rifampicin.

Only a few oral antibiotics could penetrate the prostate and achieve sufficient effective bactericidal concentration there, so

it is difficult to treat CBP (Lipsky *et al.,* 2010). Effective treatment for bacterial prostatitis depends on the delivery of a sufficient concentration of antibiotics at prostatic secretion and tissues that would inhibit bacterial growth there or eradicate them (Wagenlehner, *et al.* 2005). In addition, therapeutic schemes of CBP became more complex with the emergence of multidrug resistance (MDR) and/or ESBLs-producing bacteria, biofilm-producing bacteria and the shift in bacterial etiology.

Clinicians should consider local drug resistance patterns in selecting antibiotics that should be based on culture results. The evaluation of microbiological characteristics for each of CBP patients is essential to perform a correct, appropriate, and personalized treatment schedule (Cai *et al.,* 2011).

Because quinolones have unique pharmacokinetic properties, broad antibacterial spectrum and high activity especially against the Enterobacteriaceae (Robert *et al.,* 2001) the coverage of the quinolone class was significantly expanded by the development of fluoroquinolones, which considered as a breakthrough since they recorded a much broader spectrum of activity and improved pharmacokinetics compared to the first-generation quinolone (Adjei *et al.* 2006). Fluoroquinolones have become a common first-line agent, Ciprofloxacin in particular has been one of the most widely used fluoroquinolones for bacterial prostatitis treatment (Lipsky *et al.,* 2010). Different strategies were developed by researchers to improve the therapeutic effectiveness of ciprofloxacin and overcome the resistance issue, of them, targeting ciprofloxacin more precisely to the site of infection by using carriers. Ciprofloxacin–nanoparticles conjugation, ciprofloxacin nanoencapsulation, loading ciprofloxacin in liposomes, and polymeric nanoformations are among the different promising nanotechnology-based approaches to overcome ciprofloxacin resistance (Yayehrad, *et al.,* 2022).

In conclusion, this work targeted mainly to isolate, identify the prevalent causative bacteria for CBP in Egypt from CBP diagnosed patients in Kasr Al-Ainy hospital, Cairo. Also, local drug resistance patterns based on culture results was determined to achieve better treatment. isolates of Genus *Enterobacter sp.* represented the most common bacterial pathogen in tested EPS of CBP diagnosed patients, of them *Enterobacter cloacae* was the most predominant.

Currently, the primary medication in our treatment toolbox is fluoroquinolone (ciprofloxacin). However, the penetration of ciprofloxacin to the prostate needs to be improved especially when biofilm is present for effective treatment of CBP. Strain *E. cloacae* MAG-06 isolated in this work was chosen as the prevalent infective CBP causing bacterium for a second part of this study titled "Fabrication and application of targeted ciprofloxacin nanocarriers for the treatment of chronic bacterial prostatitis " that is published in International Journal of Pharmaceutics: X (https://doi.org/10. 1016/ j.ijpx.2024.100247).

**Declarations:**

**Ethical Approval and Consent to Participate:** Protocols for collection of samples as well as the experiment plan and all methods were performed in accordance with the guidelines and regulations of Kasr Al-Ainy hospital and approved by the institutional ethical committees. Written informed consent was obtained from participants or parents prior to their involvement.

**Competing interests:** The authors have no competing interests to declare that are relevant to the content of this article.

**Availability of Data and Materials:**All data generated or analyzed during this study are included in this published article. The nucleotide sequence of 16S rRNA gene sequences of isolated strains MAG-06, MAG-07, MAG-08, MAG-09 and MAG-10 reported in this study has been deposited in the GenBank (https://www.ncbi.nlm. nih. gov/genbank/) under the accession numbers OK087614, OK087615, OK087616,

OK087617 and OK087618 respectively.

**Authors' Contributions:** Magda M. Mehanni had made substantial contributions to the conception and design of the study, the acquisition, analysis and interpretation of data and had been responsible for critically drafting and revising the manuscript for all critical intellectual content. Ossama M. Sayed, Ahmed O. El-Gendy, Walaa G. Hozayen and Sahar I. Mohamed. had made substantial contributions to sample collections and experiments. All authors read and approved the final manuscript. All authors have read, reviewed and agreed to publish a version of the manuscript.

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## RABIC SUMMARY

**عزل، توصيف، ونمط حساسية المضادات الحيوية للبكتيريا السالبة لصبغة الجرام المسببة لالتهاب البروستاتا البكتيري المزمن**

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التهاب البروستاتا البكتيري (BP) هو عدوى في غدة البروستاتا، والذي قد يكون حادًا (ABP) أو مزمنًا (CBP).يؤثر CBP بشكل سلبي على جودة حياة المريض وقد يؤدي بدوره إلى الوفاه إن لم يُعالج بشكل صحيح. في هذه الدراسة، تم استخدام إفراز البروستاتا المستحث من 10 مرضى مصابين بالتهاب البروستاتا البكتيري المزمن في مستشفى قصر العيني بمصر لفصل البكتيريا الشائعة المسببة لـه. أظهرت النتائج أن البكتيريا السالبة لصبغة جرام كانت هي السائدة حيث تم الحصول على 54 عزلة من البكتيريا السالبة لصبغة جرام من إجمالي 62 عزلة. تم تصنيف عزلات البكتيريا السالبة لصبغة جرام وفقًا لنتائج اختبار الأكسيداز وقدرتها على تخمير اللاكتوز إلى ثلاث مجموعات. احتوت المجموعة الأكبر منها على 38 عزلة من البكتيريا السالبة للأكسيداز والقادرة علر تخمر للاكتوز وتنتمي إلى مجموعة ال *Enterobacter cloacae* . احتوت المجموعة اللإيجابية للأوكسيديز والسالبة في تخملر اللاكتوز على 9 عزلات بينما احتوت المجموعة السالبة لكل الأكسيديز وتخمر اللاكتوز على 7 عزلات. تم تعريف عزلات ممثلة لكل مجموعة جينيا من خلال دراسة تشابه تسلسل حمض الرايبوز النووي 16 S بوصفها سلالة من *Pseudomonas stutzeri* و *Providencia stuartii* على التوالي. أظهرت نمط حساسية المضادات الحيوية للعزلات المختارة أن جميعها كانت حساسة للسيبروفلوكساسين يليه الأمبيسيلين بينما كانت المقاومة للسيفاكلور والأزتيريونام سائدة. سجلت المضادات الحيوية الأخرى التي تم اختبارها أنماط حساسية مختلفة. والخلاصة أظهرت الدراسة شيوع بكتيريا ال *Enterobacter cloacae* كمسبب رئيسي للإلتهاب البكتيبري المزمن للبروستاتا.