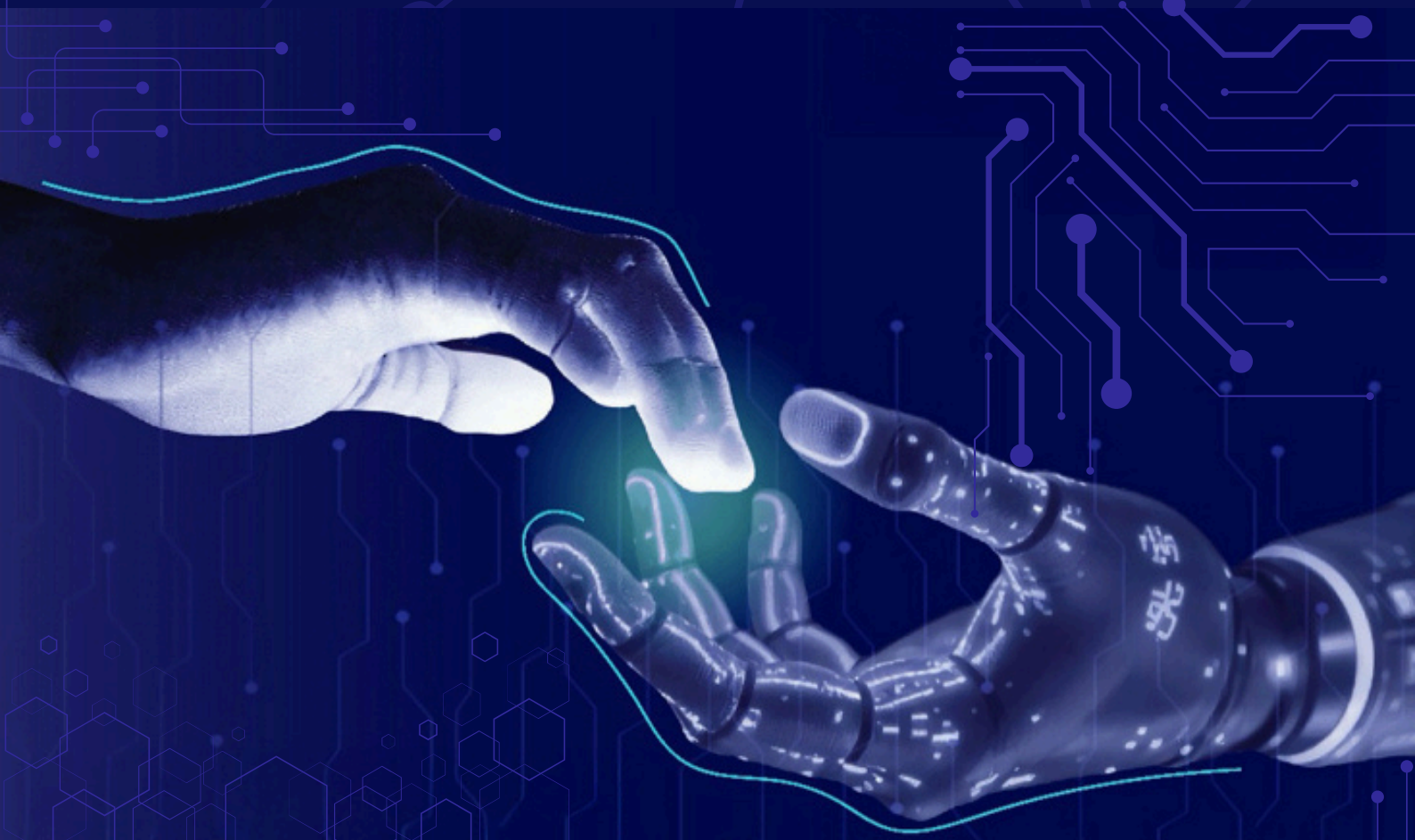
XV. International Scientific Congress of Pure, Applied and Technological Sciences

MINAR Congress



المؤتمر العلمي الدولي الخامس عشر للعلوم الصرفة والتطبيقية والتكنولوجية

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2025

XV. Uluslararası Fen, Uygulamalı ve Teknolojik Araştırmalar Kongresi

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المؤتمر العلمي الدولي الخامس عشر للعلوم الصرفة والتطبيقية والتكنولوجية

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PREFACE

XV. International Scientific Congress of Pure, Applied and Technological Sciences was organized by Igdir University in collaboration with Rimar Academy. The primary objective of this event was to compile and disseminate valuable scientific knowledge and make a meaningful contribution to the future.

A substantial number of researchers from both local and international backgrounds demonstrated their interest in this conference. The scientific committee meticulously reviewed the submissions and ultimately accepted a select group of applicants—50 in total—of whom 45 were approved by the scientific committee.

The core of this conference was the presentation of 40 full research papers, while the remaining articles and research findings are set to be featured in forthcoming issues of the MINAR Journal.

I would like to extend my sincere appreciation to all the contributors and scholars who played an essential role in making this conference a resounding success. Your dedication and valuable contributions are deeply respected and acknowledged.

Editor-in-Chief
Prof. Dr. Ghuson H. MOHAMMED

INDEX

Production of Slime Layer and Some Antibiotic Resistance Enzymes by Escherichia Coli and Klebsiella Pneumoniae Isolates

Najlaa Nabhan Yaseen [1]
Maryam Kamel Mohammed [2]
Dimah Nazar Faraj [3]

Abstract

One of the crucial public health problems worldwide is the urinary tract infections (UTIs) that are derived from uropathogenic bacteria (UPBs). Slime layer is known to have the ability to permit bacteria to achieve smooth surfaces attachment like catheters and prosthetic implants which in turn, facilitate biofilm formation and may cause lethal infections. On the other hand, Extended-spectrum beta-lactamase (ESBL) production is considered a growing concern among UPBs due to the limiting of the treatment options and contributes to resistance toward antibiotics. The principal study's point is the finding out the slime layer and ESBL production in *Escherichia coli* and *Klebsiella pneumoniae* of uropathogenic origin. Ten ready isolates (five isolates for each bacterial type) are gained from Department of Biology, College of Science, University of Baghdad. Disk diffusion method is employed to detect their antibiotic susceptibility towards Ciprofloxacin (CRO 10µg), Ceftazidime (CAZ 30µg) , Imipenem (IPM 10µg), Meropenem (MEM 10µg), Amoxicillin/ clavulanate (AMC 20/10 µg) and Aztreonam (ATM 30µg). Congo Red method is used to detect the production of slime layer for both types of bacteria. Black colonies mean slime layer production and pink colored colonies are not productive. Results show that all the tested bacterial isolates had ability to form slime layer even in different degrees. By using disk replacement method, ESBL production is detected. Results showed that two isolates (40%) of both *Escherichia coli* and *Klebsiella pneumoniae* are ESBL producers, while (60%) of both types are unable to produce these enzymes.

Keywords: *Disk Replacement Method, Escherichia Coli, Esbls, Klebsiella Pneumoniae, Slime Layer.*

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[1] College of Science, University of Baghdad, Iraq najlaa.nabhan@sc.uobaghdad.edu.iq
[2] College of Science, University of Baghdad, Iraq
[3] College of Science, University of Baghdad, Iraq

Introduction

Escherichia coli is a member of Enterobacteriaceae family that lives in human gastrointestine and warm-blooded animals as a gut microbiota (1). E. coli is a Gram-negative, non-sporulating, rod-shaped, facultative anaerobic (2). On the other hand, Klebsiella pneumonia is a commensal in human intestines, an opportunistic pathogen that is accountable for causing meningitis, lobar pneumonia, septicemia, urinary tract infections besides community and health care settings pyogenic infections (3,4).

Urinary tract infections (UTIs) are among the highly familiar infections worldwide. Uropathogenic Escherichia coli (UPECs) are the chief causal factor of UTIs. K. pneumoniae is the 2nd most common uropathogen after E. coli (5). UPECs adhere to epithelium of the bladder, followed by their urothelial epithelial cells invasion wherein their replication can occur to form biofilm of aggregated bacteria. This invasion can easily lead UPECs to establish and persist within urinary tract (6). Some bacteria can made enzymes known as Extended-spectrum beta-lactamases (ESBLs). They resulted in ineffectiveness of some antibiotics, that cause treatment of bacterial infection seriously harder. Treatment with full recommended course with correct dose is a cardinal issue. Even though, multiple bacteria construct ESBLs, they are particularly created by E. coli. Klebsiella, in addition can produce them (7). Members of this family that are ESBL-producing have a principal concern as they arise acquired infections in hospitals and community (8). Plasmid genes are accountable for ESBLs encoding, besides carrying genes of resistance to other antimicrobial agents (9). Layers of slime are amorphous and inaccordant in thickness. Relying on environment and cell type, they can be produced in diverse quantities. Protection of bacterial cells can be applied by these layers from dangers of the environment like desiccation and antibiotics. Moreover, they assist bacterial adhering to smooth surfaces (10).

Materials and Methods:

Bacterial isolates

Five isolates of E. coli and five K. pneumoniae are obtained from Biology Department, College of Science, University of Baghdad. Isolates are activated using nutrient broth and cultured on MacConkey agar and Nutrient agar (for both bacteria) in addition to use EMB for E. coli isolates.

McFarland is ready gained. It includes barium chloride (1%) and sulfuric acid (1%), which is approximately equals to 1.5 x10^{8} CFU/ml. It is used to prepare bacterial inoculum.

Detection of slime layer production

Production of slime layer is done by using Congo Red Agar which is prepared from the following components (11): Mueller-Hinton broth (21g/L), Agar-agar (15g/L), Sucrose (38g/L). whole ingredients are solvated in 900 ml of D.W. and autoclaved sucrose is sterilized by filtration by using 0.22µm filter unit then added to ingredients. However, Congo red stain (0.8 g) is dissolved in 100 ml of D.W. and autoclaved at 121°C/15 pound/inch to about 15 minutes, separately, then it is added to other ingredients after agar cooling to 55°C and poured in sterile Petri dishes.

Detection of some antibiotic resistance enzymes

Disk replacement method is performed to find out the Extended- spectrum Beta-lactamase (ESBL) production. It is conducted according to that described by Al-Jasser (2006) (12). Amoxicillin/ clavulanate disks (two instead of three) put in a plate of Mueller-Hinton that is previously inoculated with the test organism. Removal of these discs after putting for one hour at room temperature is conducted and replacement on the same spot by disks containing Ceftazidime and Aztreonam is done. Control these antibiotics discs (Ceftazidime and Aztreonam) are concomitantly placed at least 30 mm from these locations.

Increasing of a zone ≥5 mm for the discs that replaced Amoxicillin/clavulanate disks indicates a positive result for ESBL production when compared to the control disks that are placed alone immediately on inoculated plates of Mueller Hinton. By the assistance of a metric ruler, zones of inhibition are measured and recorded.

Results and Discussion

Results demonstrated that resistance percentage of E. coli isolates are 40%, 100% towards Ciprofloxacin and Ceftazidime respectively, whereas, full susceptibility (100%) to Imipenem and Meropenem is observed. Besides, K. pneumoniae isolates showed 100% resistance towards Ceftazidime and 80% of resistance towards Ciprofloxacin, with full susceptibility (100%) to Imipenem and Meropenem. Results of sensitivity test for each type of tested bacteria are compared with Standard inhibition zones according to CLSI (2023) (13) is shown in figure 1 and figure 2..

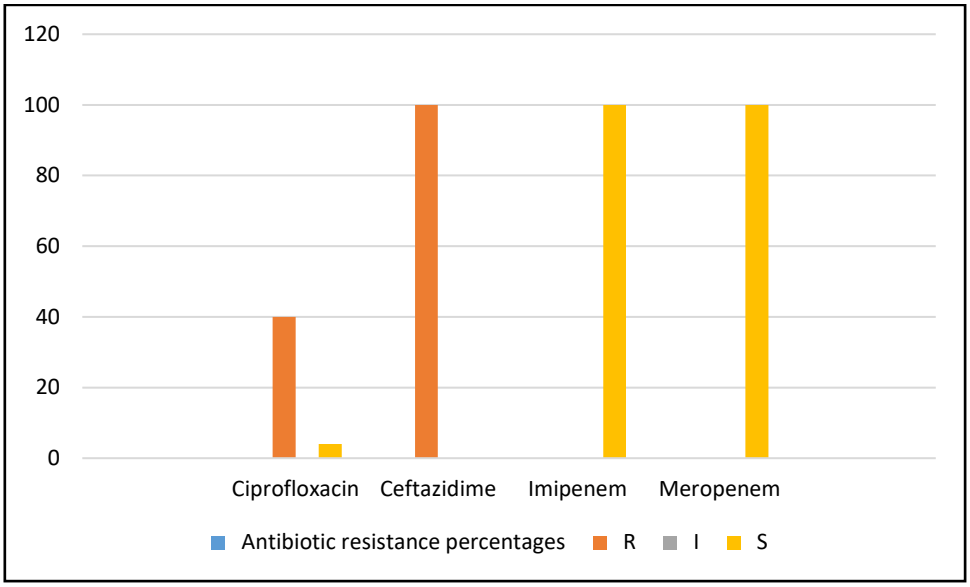

Figure 1: Sensitivity test for tested E. coli isolates towards Ciprofloxacin, Ceftazidime, Imipenem and Meropenem.

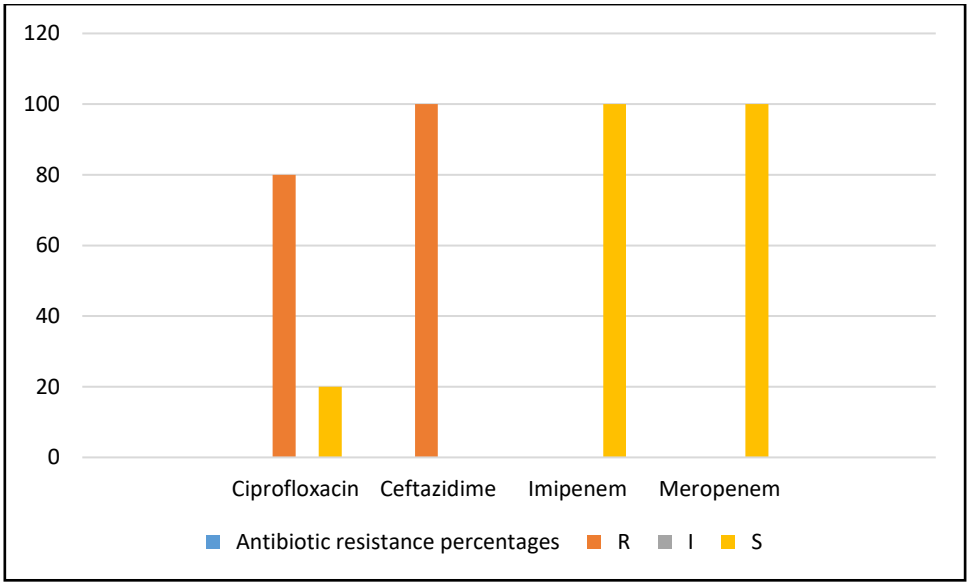

Figure 2: Sensitivity test for tested K. pneumoniae isolates towards Ciprofloxacin, Ceftazidime, Imipenem and Meropenem.

Every single isolates of E. coli is susceptible to ATM while all of the isolates (100%) are found to be resistant to CAZ. Also all isolates of K. pneumonia showed susceptibility to ATM, while (80%) are found to be resistant to CAZ. Among 10 isolates two E. coli isolates (E2 and E4) (40%) are found as ESBL producers and two K. pneumoniae (K4 and K5) (40%) are found as ESBL producers. As appeared in figures 3,4,5,6.

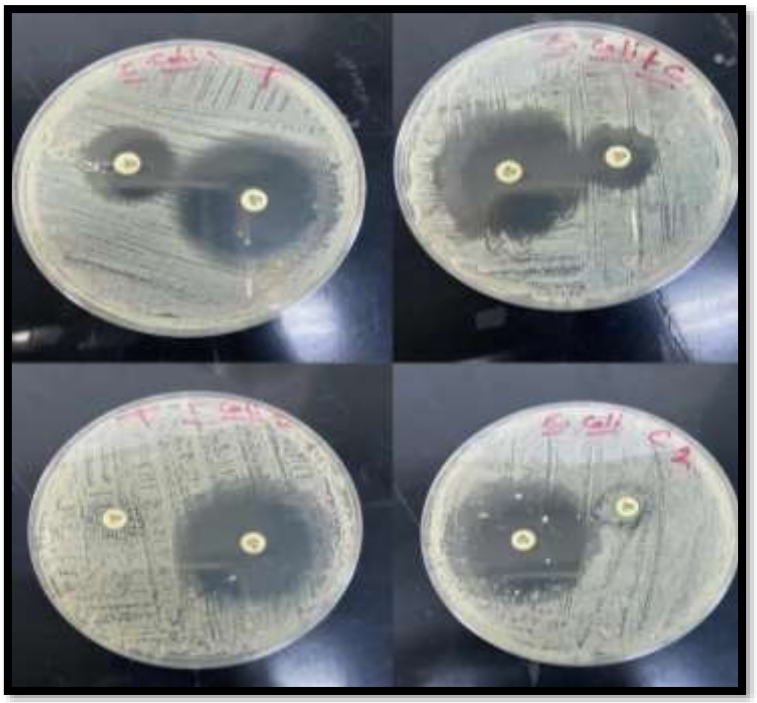

Figure 3: Production of ESBL by E. coli 1,2 isolates (on the left) compared with control same isolates (on the right).

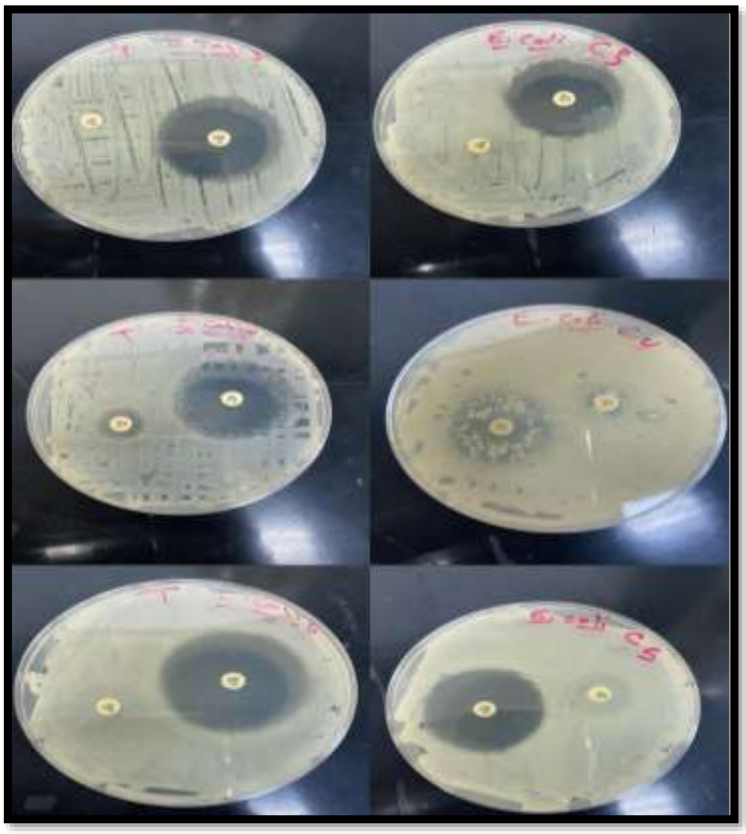

Figure 4: Production of ESBL by E. coli 3,4,5 isolates (on the left) compared with control same isolates (on the right).

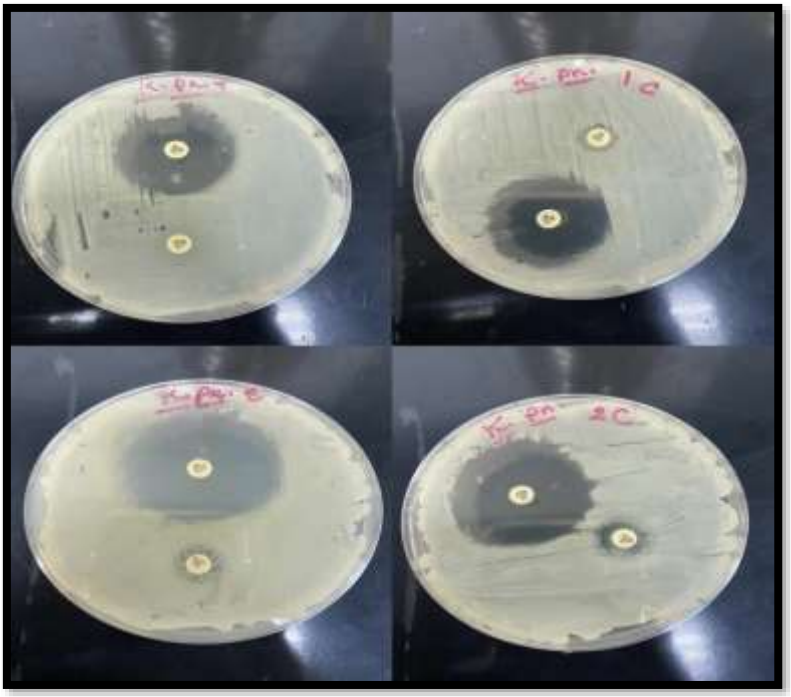

Figure 5: Production of ESBL by K. pneumoniae 1,2 isolates (on the left) compared with control same isolates (on the right).

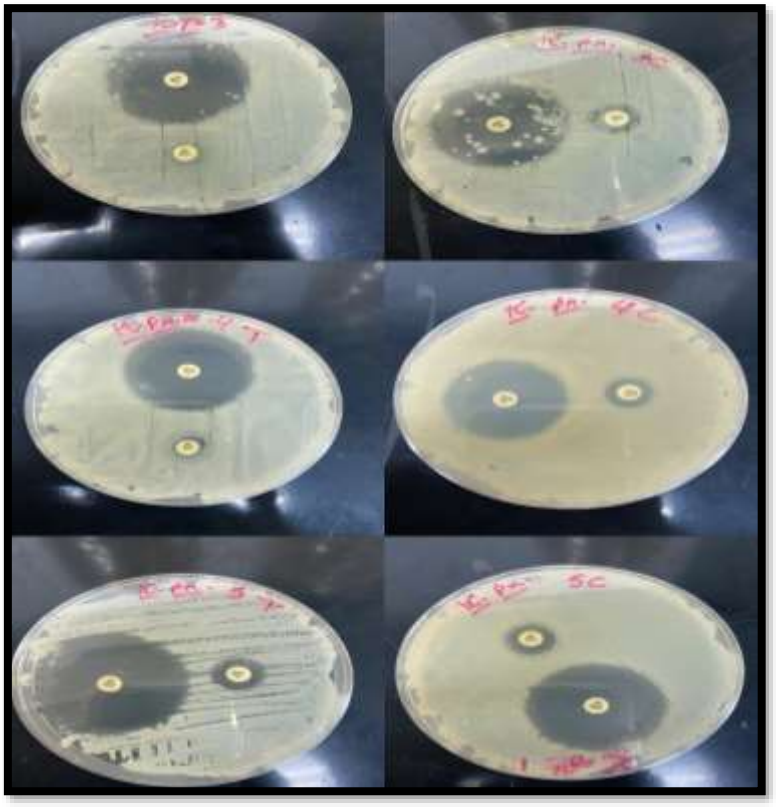

Figure 6: Production of ESBL by K. pneumoniae 3,4,5 isolates (on the left) compared with control same isolates (on the right).

For Gram-negative bacteria such as E. coli, both outer membrane that impede molecules permeability in addition to efflux pumps expression which reduce antibiotics intracellular concentration are the main contributors to the intrinsic resistance of bacteria (14). K. pneumoniae is observed to arise resistance towards antibiotics further easily than other bacteria via Extended Spectrum β-Lactamase (ESBLs) and Carbapenemase production. The prime predominant factor of antimicrobial resistance risk is the exposure of antibiotic. Antibiotics with intensive and prolonged usage are considered to be the principal contributors in the disclosure and highly resistant bacteria spreading (15). Gram-negative ESBLs producers represent significant challenge in infection management. The threat behind these organisms colonization or infection is ascribed to admission of intensive care unit, prolonged hospital stay, urinary and arterial catheterization, in addition to antibiotics exposure (16). Therapeutic options for the ESBL producers related infections have also become progressively limited. It is demonstrated an increase in ESBL producers occurrence among Enterobacteriaceae members isolated from clinical specimens (17).

Results of slime layer production

The results of the current study reveal that 100% of both bacterial isolates are slime layer producers, as they had the ability to form black colonies, but they ranged between strong, moderate and weak slime layer producers according to the density of black colonies formed on Congo red agar. Results are shown in figure 7 and 8.

Figure 7: Slime layer production by E. coli isolates. Black colonies indicates the ability of these isolates to form slime layer.

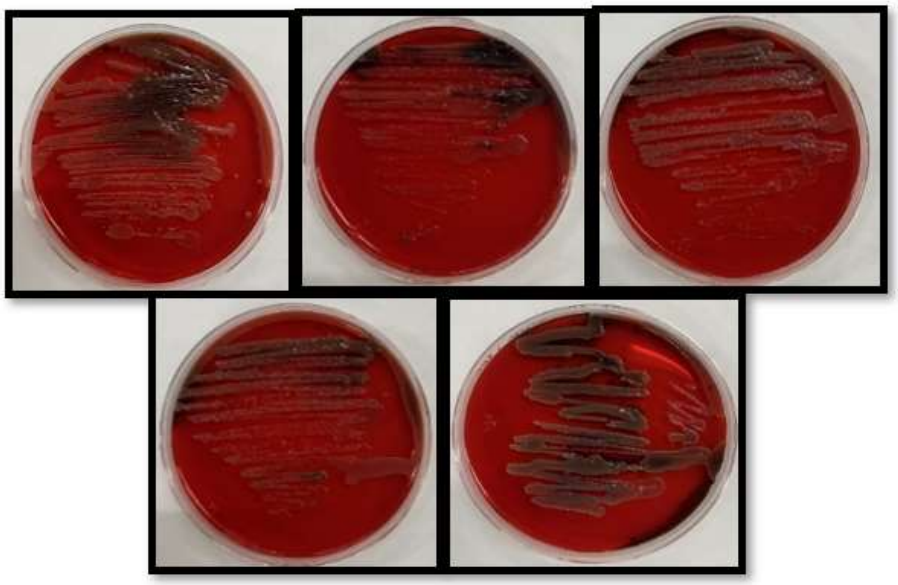

Figure 8: Slime layer production by K. pneumoniae isolates. Black colonies indicates the ability of these isolates to form slime layer.

From current results, it is clear that there isn't any isolate couldn't produce the slime layer even though they differed in the strength of its production. Colonies with black (or grey to black) color are considered as strong slime layer producers, red colonies are considered as non-slime layer producers (18). These results can indicate that both the bacterial types of uropathogenic origin can produce slime layer which, in turn, aid in the biofilm formation that protect bacteria from adverse conditions (19). Biofilm is a surface sticked bacterial cells embedded within extracellular polymeric substances matrix (20). It is previously observed that the most frequent pathogen is E. coli, follows by K. pneumoniae. They result in high UTIs percentage and can cause symptomatic UTIs (21). Bacteria can withstand adverse conditions by forming biofilm, even in the presence of antibacterial agents. These results are in accordance with previous results, which found that E. coli and Klebsiella are the predominant biofilm producers in the catheterized besides noncatheterized UTI patients (22,23,24). Previously, it is revealed that biofilm production and multiple antibiotics resistance have a vigorous correlation where MDR phenotypes are 100% biofilm producing strains (25,26,27). The cells proximity inside biofilm enable plasmids exchange, that may contribute to the spreading of antibiotic resistant strains (28).

Conclusion

E. coli and K. pneumoniae isolates showed excessive resistance to cephalosporins, and full sensitivity to carbapenem which can be a good choice to treat UTI infections by these bacteria. High correlation between slime layer production (biofilm formation) and antibiotic resistance (especially cephalosporins) is found and 40 % of both bacteria are ESBL producers that confer resistance to antibiotics. A crucial UTI virulent mechanisms is the thought to be the production of biofilm.

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