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Detection antibiotic susceptibility and biofilm form in clinical isolates of *E. coli* and *Klebsiella* spp

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Abstract

The study aim to detection antibiotic susceptibility and biofilm formation in clinical isolates of *E. coli* and *Klebsiella* spp. It was collected 264 different clinical specimens (193 urine,18 stool ,10 wound swabs ,4 vaginal swabs, 8 abscess ,5 bronchial liquid ,5 blood and 21 sputum . The results of study appeared 67 isolates (54 *E. coli* 54 and *Klebsiella* spp. 22) were isolated from 264 specimens .The result showed antibiotic susceptibility test a high resistance (100%) of enterobacterial isolates (*E. coli* and *Klebsiella* spp) were to ampicillin fo followed by amoxicillin/clavalunic acid ,it registered 29.59% and 90.91% respectively ,while imipenem was most active antibiotic agaist these isolates ,the sensitivity of isolates to this antibiotic was recorded 77.78% and 81.82% respectively. The study showed 57(75%) of both isolates were biofilm formation *Klebsiella* spp. Registered higher percentage (81.81 %) more than E .coli (72.22%),moderate biofilm was more frequently than other type of biofilm ,it was recorded 36.84% .we concluded the prevalence of biofilm producing multidrug resistant *Enterobacterial* isolates.

Key words: Antibiotic susceptibility, Bioflim, *E.coli*, *Klebsiella* spp.

Introduction

Antimicrobial resistance in these bacteria has considerable potential effects on antibiotic use and patient outcomes because *Enterobacteriaceae* are common pathogens and common causes of various types of community- and hospital-acquired illnesses.

Due to the scarcity of antimicrobials and the lack of data supporting their effectiveness, treating infections brought on by MDR and XDR *Enterobacteriaceae* is difficult. (Rodriguez-Bano *et al*., 2018). Significant members of the *Enterobacteriaceae* family, *Klebsiella pneumoniae* and *Escherichia coli* cause a wide range of illnesses, including pneumonia, UTI, bacteremia, cystitis, wound infections, etc. The major causes of urinary tract infection and bloodstream infection, respectively, are *E. coli* and *K. pneumoniae.* (Bitsori *et al*., 2019)

The great majority of bacteria that belong to the order Enterobacterales contain characteristics that can confer their virulence and pathogenicity or phenotypes. These characteristics can lead to severe health concerns, such as the development of multi-drug resistance and/or biofilm. (Amaretti *et al*., 2020)

The production of biofilms by bacteria is a one of a kind form of protection against the harmful effects of environmental factors. During this mode of growth, bacterial cells become structurally structured, and their resistance to a variety of adverse conditions is significantly increased. (Oh *et al*., 2016), Extracellular polymeric substances (EPS), which simultaneously offer tolerance to desiccation, oxidative stressors, and exogenous antimicrobials, provide support for the higher-order structure of biofilms. (Flemming *et al* .,2016), The Enterobacterales, which includes *Escherichia coli* and *Klebsiella pneumoniae*, are distinguished by a significant trait known as biofilm development (Nielsen *et al* .,2018).

The majority of nosocomial infections, including pneumonia, urinary tract infections, and infections connected to medical devices, are caused by biofilm producers (Cangui-Panchi *et al*.,2022) Once we have a better understanding of the connection between biofilm formation, the presence of virulence genes, and the distribution of antibiotic resistance in strains of *Enterobacteriaceae* that have been linked to urinary tract infections, we will be able to develop more effective strategies for the prevention and management of urinary tract infections (Donelli, and Vunotto ,2014). The capacity of bacteria to build biofilms makes them more resistant to drugs and the natural defensive mechanisms of the body. Nosocomial infections are caused by Gram-negative bacteria that have developed resistance to antibiotics and the ability to build biofilms. This type of bacteria poses a very dangerous risk (Oyardi *et al*.,2023).

MATERIALS AND METHODS

A-Isolation and identification of isolates

It was collected264 different clinical specimens (193 urine,18 stool ,10 wound swab ,4 vaginal swabs, 8 abscess ,5 bronchial liquid ,5 blood and 21 sputum .Specimens cultured on MacConkey agar and blood agar .Isolates were identified based on Morphological and biochemical tests(Macfaddin,2000) ,the diagnosis was confirmed using API 20E and VITEK 2 system(Biomerieux,France)

B-Antibiotics susceptibility

This test was performed according to the Kirby Bauer method on Mueller Hinton agar (BD&BBL, USA), in accordance with the methods established by CLSI(2020). The following antibiotic disks were used: Ampici llin 25 µg, Nalidixic acid 10 µg, Amikacin 10 µg, Amoxicillin + Clavulanic acid 30 µg ، Ciprofloxacin 10 µg, Imipenem 10 µg, Gentamycin 10 µg ،Ceftazidime 30µg, Cefotaxime, 30 µg, Ceftriaxone 30 µg, Trimethoprime 5 µ، Aztreonam 30 µg, Tetracycline 10 µg, Meropenem 10 µg, Cefepime30 µg, Cefoxitin 30 µg, (Bioanalyse, Turkey).

C- Detection Bioflim

Biofilm formation was detected using the 96-hole sterile titer plate method, as stated in Fusco *et al.*, (2017)

RESUITS

A. Antibiotic susceptibility

The results showed, that ,54 and 22 isolates of *E. coli* and *Klebsiella* Spp. Was isolates form 264 different clinical specimens .

The test of antibiotic susceptibility appeared showed (58.8%) of E. coli bacteria were resistance for activity of antibiotics that used, (29.4%)of which were sensitive, while 11.81% get moderate response for antibiotics. Also, noted all isolated E. coli resist for ampicillin, and (92.59%) of which resist for amoxicillin/ clavulanic acid, in other hand (100%) and (70.37%) were sensitive to imipenem and gentamycin respectively. Furthermore (25.93%) were had moderate response for Aztreonam.

Table (1): Antibiotics activity against *E. coli* isolates (No=54)

Antibiotics	Resistance		Intermediate		Sensitive	
	No.	%	No.	%	No.	%
Amoxicillin/ clavulanic acid	50	92.59	2	3.70	2	3.70
Ampicillin	54	100	0	0.00	0	0.00
Aztreonam	29	53.70	14	25.93	11	20.37
Cefoxitin	30	55.56	11	20.37	13	24.07
Cefotaxime	42	77.78	6	11.11	6	11.11
Ceftazidime	49	90.74	3	5.56	2	3.70
Cefepime	41	75.93	4	7.41	9	16.67
Imipenem	7	12.96	5	9.26	42	77.78
Ceftriaxone	34	62.96	6	11.11	14	25.93

Amikacin	8	14.81	10	18.52	36	66.67
Gentamycin	10	18.52	6	11.11	38	70.37
Ciprofloxacin	15	27.78	10	18.52	29	53.70
Trimethoprim	49	90.74	2	3.70	3	5.56
Tetracycline	40	74.07	2	3.70	12	22.22
Nalidixic acid	41	75.93	10	18.52	3	5.56
Meropenem	9	16.67	11	20.37	34	62.96
\CalX^2=406.2 TabX^2= 43.77 DF= 30 p. value < 0.01**						

A.

B. The results showed (41.76%) of Klebsiella spp bacteria were resistance for all of antibiotics that used, (46.02%) were sensitive, (12.5%) get moderate response for antibiotics. All isolated these bacteria were resist to a Ampicillin, and (90.91%) of which resist for amoxicillin/clavulanic acid, in other hand (81.82%) of which were sensitive to meropenem, and (77.27%) sensitive to gentamycin.(31.82%) were had moderate response for trimethoprim

C. Table 2: Antibiotics activity against *Klebsiella* Spp isolates (No=22).

Antibiotics	**Resistance**		**Intermediate**		**Sensitive**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Amoxicillin clavulanic acid	**20**	**90.91**	**2**	**9.09**	**0**	**0.00**
Ampicillin	**22**	**100.00**	**0**	**0.00**	**0**	**0.00**
Aztreonam	**5**	**22.73**	**3**	**13.64**	**14**	**63.64**
Cefoxitin	**5**	**22.73**	**5**	**22.73**	**12**	**54.55**
Cefotaxime	**9**	**40.91**	**3**	**13.64**	**10**	**45.45**
Ceftazidime	**7**	**31.82**	**1**	**4.55**	**14**	**63.64**
Cefepime	**7**	**31.82**	**3**	**13.64**	**12**	**54.55**
Imipenem	**1**	**4.55**	**3**	**13.64**	**18**	**81.82**
Ceftriaxone	**17**	**77.27**	**2**	**9.09**	**3**	**13.64**
Amikacin	**4**	**18.18**	**4**	**18.18**	**14**	**63.64**
Gentamycin	**3**	**13.64**	**2**	**9.09**	**17**	**77.27**
Ciprofloxacin	**5**	**22.73**	**2**	**9.09**	**15**	**68.18**
Trimethoprim	**10**	**45.45**	**7**	**31.82**	**5**	**22.73**
Tetracycline	**19**	**86.36**	**1**	**4.55**	**3**	**13.64**
Nalidixic acid	**11**	**50.00**	**4**	**18.18**	**7**	**31.82**
Meropenem	**2**	**9.09**	**2**	**9.09**	**18**	**81.82**
CalX^2=155.4 TabX^2= 43.77 DF= 30 p. value < 0.01**						

B. Bioflim formation

The findings of this study revealed 57 (75 %)out of 76 isolatesof *Ecoli* and *Klebsiella* Spp had ability to form biofilm *Klebsiella* Spp registered higer percentage(81.81%) more then E coli (72.22%), the results showed the isolates ability to form moderate biofilm was more frequent than other type of biofilm it was recorded (36.84%) and *E coli* registered (37.03%) more than *Klebsiella* Spp.

Table (3): Number and percentage of isolates with Bioflim formation

Bacteria	Total	No &(%)of bioflim formation	No (%)of isolates with strong bioflim	No (%) of isolates with moderate bioflim	No (%) of isolates with weak bioflim	No (%)of isolates without bioflim formation
E. coli	54	39(72.22)	13(24.07)	20(37.03)	6(11.11)	15(27.77)
Klebsiella spp	22	18(81.81)	5(22.72)	8(36.36)	5(22.72)	4(18.18)
Total	76	57(75)	18(23.68)	28(36.84)	11(14.47)	19(25)

Discussion

The findings of this investigation demonstrated that *E.coli* is isolated from clinical specimens more frequently than *Klebsiella* spp., these results were in agreement with Yinnon *et al*. (1996), who found that *Klebsiella* is second only to *Escherichia coli* in nosocomial gramme-negative bacteremia, and a study by Najjuka *et al*., (2016) found growth from stool occurred in 636 (87%) samples, of which 620 (97%) were *E. coli* and 16 (3%) were *K. pneumoniae*. Growth from urine occurred in 349 (49%) samples, of which 310 (89%) were *E. coli* and 39 (11%) were *K. pneumonia*. The antibiotic susceptibility results found that higher resistance (100%) appeared in both isolates of *E. coli* and *Klebsiella* spp. Were against ampicillin followed by amoxicillin and clavulanic acid, it registered 92.59% and 90.91%, respectively.

These results were similar to the findings of Shatalov (2015), which appeared to show almost 100% resistance of *E. coli* and *K. pneumoniae* to ampicillin, and the resistance of isolates to amoxicillin and clavulanic acid was 74.1% and 93.3%, respectively. The study of Jabar and Abid (2021) shown that 98% of Klebsiella spp isolates were resistant to ampicillin, while 96% of them were resistant to amoxicillin-clavulanic acid. This could be because of the synthesis of beta-lactamases or because antibiotics were unable to reach their intended target (PBPs) (Harwood *et al*., 2000).

The formation of AmpC -lactamases, which mediated the resistance to this -lactamases inhibitor, may be responsible for the development of resistance to amoxicillin-clavulanic acid. The results showed that carbapenem (imipenem) antibiotics were more effective on two types of isolates (*E. coli* and *Klebsiella* spp.). These results were in line with the results of 2012 that showed imipenem and meropenem were the most effective antibiotics in terms of activity against *E. coli* (92.5% and 95%, respectively) and *K. pneumoniae* (87.2% and 87.2%, respectively). According to the findings, the isolates exhibited a high level of resistance to other classes of antibiotics as well, including tetracycline and nalidixic acid, in addition to being extremely resistant to beta-lactam antibiotics. MDR is defined as the inability to respond to treatment with at least one agent across three or more different classes of antimicrobials.

These findings provide further evidence that carbapenems are the most effective treatment options for illnesses caused by bacteria that are resistant to several drugs.

Escherichia coli and *Klebsiella pneumoniae* are two enterobacteria that generate substantial biofilms (Nielsen *et al*., 2018). Recent research has put forward the hypothesis that the mode of growth known as biofilm is the primary contributor to persistent infections. Biofilms have the potential to operate as a nidus, which would result in periodic planktonic showers of bacteria entering the bloodstream, which would lead to acute illnesses (Costerton *et al*., 1999). It has been found that biofilm specific resistance to antibiotics is orders of magnitude stronger than the antibiotic resistance of planktonic bacteria. (Hoyle and Costerton, 1991).

The findings of this research indicated that *Escherichia coli* isolates had a lower percentage of biofilm producing *Klebsiella* spp. These findings are consistent with the findings of the research conducted by Dumaru et al. (2019), which demonstrated a high incidence of biofilm producing *Klebsiella* spp.(77.55%) and *E. coli* (60.33%). These results were not similar to the results of Mahmood and Abdullah (2015), They discovered that the percentage of *Escherichia coli* and *Klebsiella pneumoniae* that led to the formation of biofilm was (53.6%) and (52.9%), respectively, from urinary tract infections (UTIs) and (58.8%) and (62.5%), respectively, from diabetic foot infections. According to the findings of this investigation, the type of biofilm that was found most frequently among the isolates was the moderate type; these findings were not in agreement with the results of Mahmood and Abdullah (2015), who reported that the robust kind of biofilm was the most common type among all of the different types of biofilm.

Alansary and Al-Saryi (2022) found that (63%) of *Klebsiella pneumoniae* can form biofilm by the TCP method, divided as: (14%) strong, (15) moderate, (34%) weak, and (37%) makers of no biofilms at all. For this reason, having knowledge of the formation of biofilms and the presence of antibiotic resistance in bacterial isolates is of the utmost relevance in order to provide patients with effective and trustworthy empirical antibiotic therapy. (Tanwar *et al*.,2014). Our findings led us to the conclusion that there is a widespread presence of biofilm producing enterobacterial isolates (such as *Escherichia coli* and *Klebsiella pneumoniae*) that are resistanttomanyantibiotics.

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