Assessment of bacteria exposure *in vitro* activity to 1st, 2nd, 3rd and 4th generation-cephalosporins and Comparison effects

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Abstract

The emergence and spread of resistance to cephalosporin generations are threatening to create species resistant to all currently available agents. Recently, we have seen the development and spread of bacteria carrying metallo-betalactamase genes that are resistant to cephalosporins (and all beta-lactams). This study was designed to comparison the effects of first, second, third and fourth- generation-cephalosporin on different bacterial species, which include: *Escherichia coli*, *Enterobacter cloacae*, *proteus mirabilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Streptococcus pneumonia* and *Staphylococcus aureus*. 11 cephalpsporins antibiotics were used in this study, which include: cefalexin, cefazolin, cephalothin, cefuroxime, cefoxitin, cefaclor, ceftibuten, cefotexime, ceftazidime, cefpirome and cefepime. kirby bauer method was used to detect the activity of these antibiotics in vitro. Results showed that cefpirome and cefepime antibiotics belonging to 4th generation cephalosporin, exhibit antibacterial spectrum effective, except *Str. pneumonia* and *K. pneumonia*. Some cases, 1st generation cephalosporin exhibit more antibacterial spectrum effective than other cephalosporin generation. In conclusion. This study indicated that insignificant influence among four cephalosporins generation on different bacterial species. Although, cephalosporins antibiotics have variant activity against different bacterial species, but the resistant development among bacteria become the public problem during the past 2 decades.

تقييم نشاط تعرض البكتريا خارج الجسم للجيل الاول والثاني والثالث والرابع لمضاد Cephalosporin ومقارنة التأثيرات

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الخلاصة

ان ظهور وانتشار المقاومة لأجيال المضاد الحيوي Cephalosporin يهددان بخلق انواع بكتيرية مقاومة لجميع العوامل المتوفرة حاليا". مؤخرا"، لاحظنا تطور وانتشار للبكتريا الحاملة لجينات metallo-betalactamase genes والتي تعطي لهذه البكتريا صفة المقاومة لل Cephalosporin بالأضافة الى مقاومتها جميع المضادات من نوع beta-lactams. هذه الدراسة صممت لمقارنة تأثيرات الجيل الاول والثاني والثالث والرابع لمضاد cephalosporin على انواع مختلفة من البكتريا والتي *Escherichia coli*, *Enterobacter cloacae*, *proteus mirabilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Streptococcus pneumonia* and *Staphylococcus aureus*. تم استخدام 11 نوع من المضادات موضوع بحثنا، حيث شملت cefalexin, cefazolin, cephalothin, cefuroxime, cefoxitin, cefaclor, ceftibuten, cefotexime, ceftazidime, cefpirome and cefepime اجري فحص الحساسية للمضادات داخل المختبر بأستخدام طريقة Kirby bauer للكشف عن فعالية هذه المضادات. اظهرت النتائج ان مضادات الجيل الرابع لل- Cefpirome and cefepime كانتا ذات طيف فعال ومضاد لجميع الانواع البكتيرية المستخدمة في هذا البحث عدا *Str. pneumonia* and *K. pneumonia*. وبالمقارنة فأن معظم الانواع البكتيرية اظهرت مقاومتها لمضاد Cefazolin والذي يعود للجيل الاول. نتيجة لذلك، فأن هذه

الدراسة خلصت الى عدم وجود تأثير ملموس بين الاجيال الاربعة لمضاد Cephalosporinعلى الانواع البكتيرية المختلفة. وعلى الرغم من معظم البحوث تشير الى انتشار وتطور مقاومة المضادات بين البكتريا خلال العقدين الماضيين، الا ان مضادات Cephalosporin تبقى ذات تأثير متباين الفعالية على مختلف الانواع البكتيرية.

الكلمات المفتاحية : الجيل الاول والثاني والثالث والرابع لمضاد Cephalosporin، طيف مضادات البكتريا.

Introduction

Cephalosporin antibiotics belonging to β-lactam antibiotics. Their structure and function closely relate to the penicillins, and classified as bactericidal, and they have the same penicillins effect. Cephalosporins are more resistant to the β-lactamases. These extracellular enzymes produce by some Gram-negative bacteria and inactivate of penicillin antibiotics when breaking the beta-lactam ring [1]. Cephalosporins classification based on the two R- group's compounds of beta-lactam ring and pharmacological features. So that they are classified to many generation according to these characters. In recent years, most hospitals in modern country prescribe the cephalosporin antibiotics as a main part of the antibiotics formulary, because they have a broad spectrum of activity and limited side effects, so physicians are wide prescribed it [1,2]. The pharmacological and structural of cephalosporin are related to penicillin, since both have a beta-lactam ring structure that inhibit synthesis of the bacterial cell wall [3,4]. Commonly used antibiotics include the penicillin, cephalosporins, aminoglycosides, tetracyclines, chloramphenicol, erythromycin and polymyxins and the common synthetic antimicrobials are the trimethoprim, nalidixic acid and sulphonamides [5]. Cephalosporin are used to treat otitis media, staph infections, strep throat, bronchitis, pneumonia, tonsillitis, gonorrhea, some infections of skin and commonly used for surgical prophylaxis [6]. Cephalosporin antibiotics are grouped into generations according to their antimicrobial characters and categorized chronically, so they are classified into first, second, third and fourth generation. The newer generation of cephalosporin has greater antimicrobial properties on Gram negative than the previous generations. Some reports refer that Cefpirome, Cefozopran and Cefepime antibiotics belonging to 4^{th} generations of cephalosporin has greater effect against resistant bacteria [5,7]. In the final two decades, the greatest health problems mainly in hospitals are antimicrobial resistance [8,9,10]. The most common resistance mechanism in Gram-negative bacteria is β-lactamase production . The broad spectrum β-lactamase enzyme are mediated by plasmid found in *E. coli* and *K. pneumoniae* given resistance to the first cephalospon generaton [11,2]. Enterobacteriaceae has become more resistant to 3^{rd} generation of cephalosporin which is the cause of nosocomial infections [12]. Resistance of *Staph. aureus* to methicillin-Methicillin Resistant (MRSA) and *E. coli* to 3^{ed} generation of cephalosporin and fluoroquinolones are reported to be 50% or more in five out of the six World Health Organization (WHO regions) [13,10]. Garaul et al., (2012) and Jeong et al., (2016), refer that the 3^{ed} and 4^{th} generation of cephalosporins have related structure, since they have a $NR4^{+}$ group in the C / 3 of R-group position. This feature facilitates these antibiotics fast passing throgh the outer membrane of Grm-negative. To shed light on the in vitro antibacterial spectrum of the four cephalosporin

generations, the current study was done to detect that.

Material & Methods

Bacterial Isolates:

Seven of different clinical bacterial isolates were used in this study (Table-1). All these isolates were submitted to identification tests, which include: Gram stain, Oxidase, Catalase, Urease, IMVC, Coagulase and Hemolysis. In addition to detection the ability of fermentation sugars, which are lactose, glucose and mannitol using bacterial media, these include: MacConkey agar, Mannitol Salt agar and Kligler Iron agar [14,15].

Table (1):- Bacterial species

Bacterial isolates	Source
Escherichia coli	UTI
Klebsiella pneumonia	UTI
Enterobacter cloacae	UTI
proteus mirabilis	Diarrhea
Pseudomonas aeruginosa	Otitis media
Streptococcus pneumonia	Otitis media
Staphylococcus aureus	Inflamed Wound

UTI. Urinary tract infection.

Antibiotic sensitivity (disc diffusion test):

This test was performed according to (Schwalbe *et al.*, 2007; Ferraro *et al.*, 2006).

a. 3 to 5 of bacterial colonies were transfer to a tube of saline.

b. The turbidity of tube was compared and adjusted to 0.5 McFarland turbidity standard using saline or broth.

c. The plate of Mueller-Hinton agar was inoculated by dip a sterile swab into the inoculum and the excess inoculum was removed.

d. The plates were streaking by the swab all over the surface of the medium many times. Finally, allowed to dry then cephalosporin antibiotics impregnated discs with required concentration (Becton. Dickinson and company sparks-USA), (table-2).

e. All petri dishes were incubated at 35°C for 24 hours.

f. Using ruler, the inhibition zones were recorded.

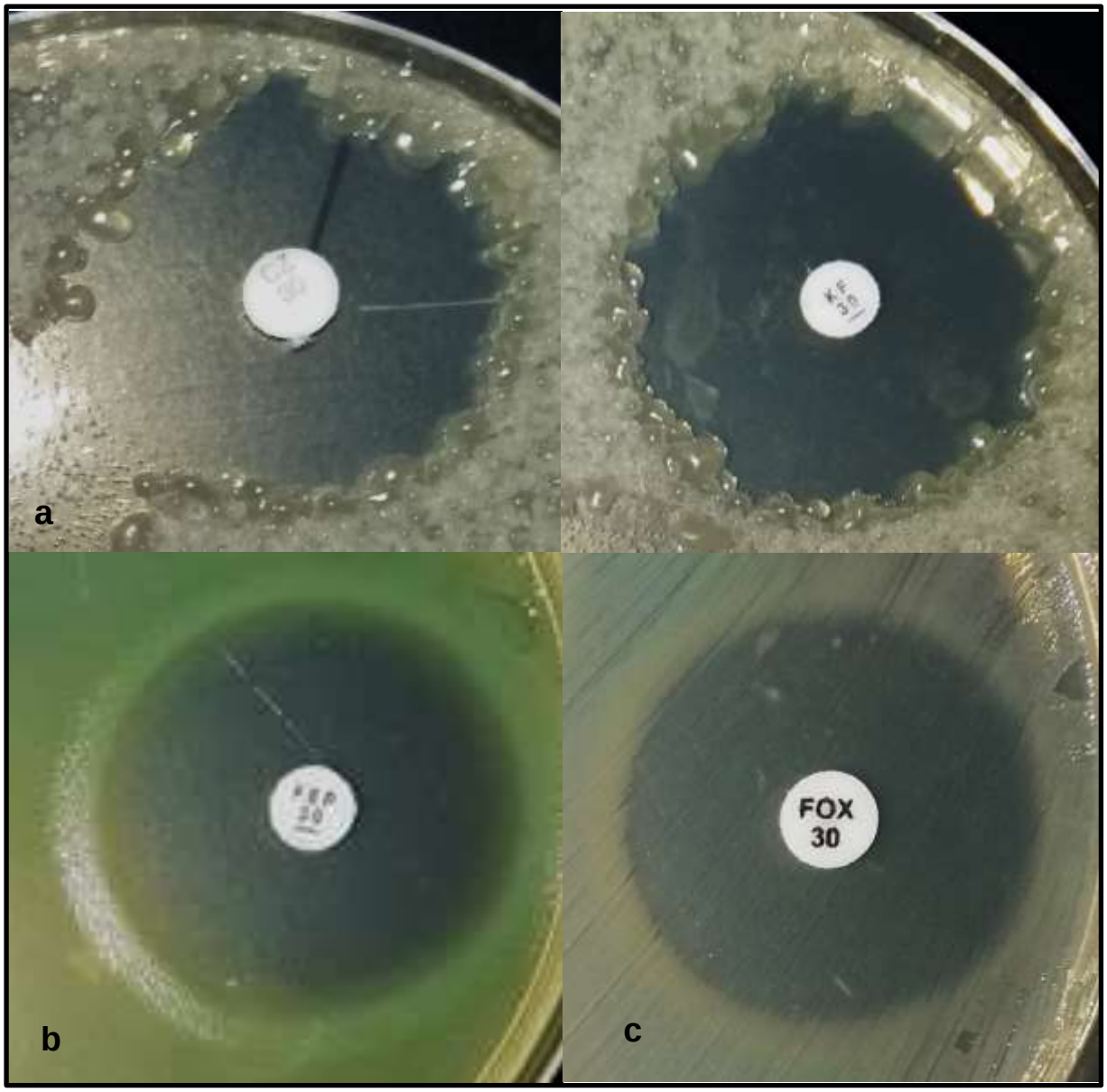
Results

Bacterial isolates were screened for their susceptibility to eleven cephalosporin antibiotics, using kirby bauer method. The antimicrobial susceptibility profiles results of the seven bacterial isolates are shown in Table-2. Results reveal that there are resistance variations among bacterial species to the four cephalosporins generations.

Table (2):- Cephalosporins susceptibility profiles results.

Generation	Cephalosporin	Bacterial species						
		E. coli	*K. pneumonia*	*E. clocae*	*P. mirabilis*	*P. aeruginosa*	*Strep pneumonia*	*Staph. aureus*
First G.	**Cefalexin**	S	R	R	I	R	S	S
	Cefazolin	R	R	R	S	R	R	S
	Cephalothin	I	R	R	S	R	R	S
Second G.	**Cefuroxime**	S	R	S	S	R	R	S
	Cefoxitin	S	S	R	S	R	R	S
	Cefaclor	S	R	R	S	R	R	S
Third G.	**Ceftibuten**	S	I	I	S	R	R	R
	Cefotexime	S	R	S	S	I	R	S
	Ceftazidime	S	I	S	S	S	R	R
Fourth G.	**Cefpirome**	S	R	S	S	S	R	S
	Cefepime	S	R	S	S	S	R	S

Figure-1, shows some pictures of cephalosporins effect. *Strep. pneumonia*, isolated from patient with Otitis, reveals large resistance to most cephalosporins antibiotics that used in this study, include all first, second, third and fourth generation, except Cefalexin antibiotic belonging to first cephalosporin generation. Then *Klebsiella pneumonia* bacteria isolated from patient with urinary tract infection, which also resistant to all used antibiotics except cefoxitin, ceftibuten and ceftazidime. While *proteus mirabilis* were sensitive to most cephalosporins antibiotics that used in this study, include all first, second, third and fourth generation, except cefalexin, then *Escherichia coli*, also sensitive to these antibiotics except cefazolin and cephalothin



Figure(1):- Some pictures show cephalosporins effect. CZ. Cefazolin, KF. Cephalothin, FEP. Cefepim and FOX. Cefoxitin. a. *Staph. aureus*, b. *P. aeruginosa* and c. *E. coli*.

Discussion

Bacterial resistant to antimicrobial agents is a main problem of concern in the final two decades [13], but in cephalosporin-resistance bacteria, there is no cross-reaction as penicillin. Occasional *E. coli* organisms may appear susceptible in vitro to cefazolin (first-generation cephalosporin) but resistant to ceftazidime (third-generation cephalosporin). When this occurs, report all cephalosporin results so clinicians do not extrapolate that the isolate is susceptible to all cephalosporins because the isolate is susceptible to cefazolin. The major cause underlying the emergence of resistance and continues to be a problem is excessive and inappropriate use of antibiotics, in spite of the existence of published guidelines and the implementation of antimicrobial administrations in many hospitals

[8,3]. The extensiveness of cephalosporins use has caused the emergence of extended spectrum β-lactamase in Gram-negative bacteria worldwide [19]. More cephalosporin antibiotics especially 3^{ed} generation are being widely used in hospitals for empirical and prophylactic therapy and as their use extends across the board more microorganisms will develop resistance to them presenting the threat of antimicrobial ineffectiveness in life threatening infections [19]. In West Africa, Okesola A. O and Makanjuola O. (2009), found that 66% *of E. coli* were sensitive to ceftazidime, 63% to ceftriaxone and 72% to cefotaxime. 55% of the klebsiella species isolated were sensitive to ceftazidime, 48% to ceftriaxone and 31 % to cefotaxime. In proteus species, 50% were sensitive to ceftazidime and ceftriaxone, 0% to cefotaxime. In this study, *Streptococcus pneumonia* reveal large resistant to cephalosporin antibiotics, and these results were agree with many studies [20,21,22,23,24]. This strain *Strep. pneumonia* is interesting, since resistant to second, third and fourth cephalosporin generations that screened in this study, but susceptible to Cefalexin belonging to fist cephalosporin generation. Iain B Gosbell and Stephen A Neville (2002); Elisabeth et al., (2010), refer that *Strep. pneumoniae* is a main bacterial pathogen. The emergence of resistance in the drugs is used to treat infections with these organisms is of major public health significance. In our study *K. pneumonia* bacteria isolated from patient with urinary tract infection, which also resistant to all used antibiotics except cefoxitin, ceftibuten and ceftazidime. These result agree with Mary et al., (2016), which concluded that *K. pneumoniae* resistance to 3^{ed} generation of cephalosporin is reported to be greater than 50% in all six (WHO regions). However, third generation of cephalosporin still effective in most bacteria; for example, uncomplicated goncoccal infections of the, rectum, urethra, or endocervix can use ceftriaxone, cefixime and ceftazidime as single dose of therapy. On the other hand, Cefepime and Cefpirome antibiotics belonging to fourth cephalosporin generations, exhibit antibacterial spectrum effective. These results also agree with [11] which refer that, cefepime and cefpirome have a good balanced antibacteral spectrum, including Grm-negative bacteria and Grm-positve cocci, these findings were consistent with our results. Also they refer that, cefpirome and cefepime show a greater effect in vitro than third generation cephalosporin because these antibiotics are more effective against Enterobacteraceae which produce class I β-lactamase which may inactivate 3^{ed} generation of cephalosporin [11,26]. cefpirome and cefepime are more active in vitro than 3^{ed} genrations of cephalosporin against Grm-positve cocci including methcillin-suscptible *Staph. aureus.* Additionally, 4th genration of cephalosporin unlike 3^{ed} genration of cephalosporin since they are active in vitro against Grm-negative baclli which produce depressed amounts of AmpC beta-lactamses [3,26]. 70% of the pathogenic bacteria or more are found in the USA hospitals are resistant to most traditional antibiotics, in spite of the development of antibiotics and introduction a new antibiotics, several bacteria are continuous in resistant to it [8,9,10].

Conclusion

This study indicated that insignificant influence among four cephalosporins generation on different bacterial species. Some cases, 1st generation cephalosporin exhibit more antibacterial spectrum effective than

other cephalosporin generation. *Strep. pneumonia* and K. *pneumonia* exhibits wide spectrum of antibiotics resistance, and this may have a new β-lactamase enzyme which hydrolysis the cephalosporin generation. Although, cephalosporns antibiotics have variant activity against different bacterial species, but the resistant development among bacteria become the public problem during the past 2 decades.

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