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The Dynamics of Antimicrobial Resistance among Enterobacteriaceae Isolates to Ceftazidime in Benghazi, Libya

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Abstract— ***Enterobacteriaceae are among the most typical pathogens, causing a broad range of community-acquired and healthcare-associated infections and associated with substantial worsening of patients' quality of life and increased mortality. Ceftazidime is a combination of the third-generation intravenously administered cephalosporin ceftazidime and the novel non-β-lactam b-lactamase inhibitor avibactam. This study aimed to explore the invitro activity of ceftazidime against bacterial isolates from various clinical samples. The samples collected from the wounds pus, burn; medical devices, tips and blood from male and female patients in the age group of 3 to 86 years with clinical characteristics suspected to be infected and are transmitted to the Microbiology Department of Al-Saleem laboratory, Benghazi, Libya between October 2021 and January 2022. The swab infection rate was higher in males than in females. The majority of the subjects (19.6%) were in the 40-49 years old group. E. coli and klebsiella spp were the most prevalent pathogens of pus swab contagion respectively. The most bacteria found were E. coli, 57 isolates (32.6%) and Klebsiella spp 18 isolates (19.6%). The susceptibility test for ceftazidime found that 30.4% of E. coli isolates were susceptible to ceftazidime. The results of this study indicate that Gram-negative bacteria are less sensitive to ceftazidime. Almost isolated strains showed a ratio of intermediate to ceftazidime. Therefore, Ceftazidime is not recommended for use as an Empirical treatment.***

Keywords- Ceftazidime, infections, intermediate susceptibility, pus swab., Benghazi

I. INTRODUCTION

Antimicrobial resistance (AMR) is the ability of a microbe to resist the effects of drugs formerly used to treat them. The term "antibiotic resistance", relates only to bacteria becoming resistant to antibiotics. Resistant microbes are more difficult to treat, requiring alternative drugs or higher doses, both of which may be more exclusive or more toxic. Microbes that are resistant to multiple antimicrobials are called multidrug-resistant (MDR); or occasionally superbugs. (2) Ceftazidime is an established third-generation, broad-spectrum cephalosporin that, like other b-lactam antimicrobials, exerts its antibacterial effect by binding to penicillin-binding proteins (PBPs) thereby inhibiting peptidoglycan crosslinking during cell wall synthesis, this leads to lysis and death of bacterial cells. (3, 4) Aim of this study was to examine the invitro activity of ceftazidime against bacterial isolates from various clinical samples**.**

II. EXPERIMENTAL

A. Study area

The specimens collected from the wounds pus, burn; medical devices, tips and blood from male and female patients in the age group of 3 to 86 years with clinical characteristics suspected to be infected and transferred to the Microbiology Department of Al-Saleem laboratory, Benghazi, Libya between October 2021 and January 2022.

B. Sampling

A total of ninety-two isolates were isolated from clinical specimens from patients included in this study. All swabs specimens were amassed aseptically and carried from patients by Stuart's, Amies media. The collected blood samples were inoculated in brain heart infusion (BHI) broth with a blood to broth ratio of 1:10 for up to 5 days. And immediately transferred to the Department of Microbiology. The age, gender, health status, drug use, and type of infection have been recorded for all included patients.

C. Cultures

Positive culture samples were inoculated onto blood agar and MacConkey agar and then incubated overnight at 37°C

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aerobically. Chocolate plates were incubated at 37 °C in microaerophilic conditions (containing 5% CO2). Moreover, 0.5 to 1 mL of the swab specimen was inoculated into a tube of nutrient broth. (12)

D. Determination of bacterial isolates

Isolation was done by multiple streaking of Bacterial colonies having diverse morphological characteristics. Bacterial colonies were picked by a loop from primary culture plates and cultured on Blood agar MacConkey agar and Nutrient agar plates, labelled and incubated at 37°C for 24 hours. To identify unknown pure bacterial cultures, we studied colony morphology and performed microscopy and genus and species-level, biochemical tests using the standard protocols of Bergey's Manual of Determinative Bacteriology. (1)

Visual investigation of the bacterial colonies shows culture positivity. The supposed organism is then defined by Gram's stain and a series of biochemical tests, including an oxidase test, catalase test, Carbohydrate utilization test, Citrate utilization test, DNAase test, urease test, coagulase test, Analytical profile index (API) 20e test and Mannitol Salt Agar. (12, 13)

E. Direct Vitek 2 AST

The supposed strains' morphologically similar colonies were determined by biochemical assays utilising the Vitek system (bioMerieux, Hazelwood, MO). In brief, the cell density of the remaining bacterial suspension was modified to a density of 0.5 McFarland after dilution in 0.45% saline; 145µL of the bacterial suspension was drawn into 3 mL of 0.45% saline solution to further adjust the bacterial cell density. Vitek cards were inoculated with suspension vials and loaded into the Vitek automated reader-incubator. (13**)**

F. Antimicrobial susceptibility testing

The sensitivity of the bacterial isolates to antibacterial drugs was performed by the disc diffusion method of the ceftazidime antibiotic. The results analyzed based on CLSI recommendations. (12) Briefly 4–6 morphologically similar bacterial colonies were collected from clinical samples using an inoculation loop and transferred into a tube containing 5 mL of broth culture, mixed gradually and carefully until a relatively homogeneous suspension was obtained, and incubated at 37 °C. A sterile, non-toxic dry cotton swab used to break a sample from the central inoculation onto a Mueller-Hinton agar plate. With the cap on, the inoculum was left to dry for 5-15 minutes. Antibiotic tablets were distributed onto the plates with sterile forceps at a distance of 15 mm from the edge and 24 mm from each other. After 24 hours of incubation at 37 °C, the diameters of the bacterial growth inhibition zones around the discs were measured to the nearest millimetre. Isolates were classified as sensitive (S), or resistant (R) using a standardized table. The inhibition area of the ruler was measured in millimetres. (12) Susceptibility testing was performed using Clinical and Laboratory Standards Institute recommendations. (13) MIC to fusidic acid was determined using Etests (BioM´erieux, AB Biodisk, Solna, Sweden) on Mueller-Hinton agar incubated for 24 hours. Disk diffusion zone sizes were determined by direct suspension of the colony to 0.5 McFarland, the suspension was inoculated with Mueller-Hinton plates with 10 µg of fusidic acid tablets (Biomerieux, AB Biodisk, Solna, Sweden), and the plate was read after incubation for 16–20 h. at 35°C. A colony suspension equivalent to 0.5 McFarland agar was inoculated with Mueller-Hinton with a 30 µg cefoxitin tablet (Oxoid, Basingstoke, UK) and lysed after 16–20 hours. MRSA was determined using a 21-mm area-size breakpoint for cefoxitin tablets. For oxacillin Etests (AB Biodisk, Solna, Sweden), 0.5 McFarland direct colony suspension was inoculated into Mueller-Hinton plates with 2.0% NaCl and lysed after 24 h incubation. Isolates with a MIC of 4.0 µg/ml were considered to be oxacillin resistant. (12)

G. Data Analysis

The data was analysed by SPSS programs version 20

III. RESULTS AND DISCUSSION

A. Distribution table of different Wound pus and blood Infection by genders

Most of the samples were collected from pus swabs 75% in males. This finding was in contrast to the study of Saleem *et al*., 2018. (8) The gender distributions of the patients with swab infection are reported in Table. 1 Concerning gender, the swab infection rate was higher in males than in females.

TABLE I Distribution table of different swabs and blood Infections by genders**.**

Gender	Frequency	Percent
Female	44	47.8
Male	48	52.2
Total	92	100.0

B. Distribution of positive cases by age groups.

The study samples included in this study were from less than 9 to 89 years old, and the majority of subjects (19.6%) were from the age group 40-49 years. This study was in contrast to what was reported in the study of Salim *et al*., 2018. [8]

TABLE II . Distribution of positive cases by age groups.

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AGE	FREQUENCY	PERCENT
0-9	11	12.0
10-19	7	7.6
20-29	17	18.5
30-39	12	13.0
40-49	18	19.6
50-59	15	16.3
60-69	6	6.5
70-79	4	4.3
80-89	2	2.2
TOTAL	92	100.0

C. Distributions of bacterial infection in various swabs specimens Collected from Patients.

The most bacteria found were *E. coli*, 57 isolates (32.6%) and *Klebsiella* spp 18 isolates (19.6%). Concerning the causative nosocomial infections in the present study. The isolates belonged to gram-negative bacteria. *E. coli* was the most prevalent, and *Klebsiella* spp. was the second most. This study is similar to the research of Ibrahim et al. (2012). (8, 9)

TABLE III. Distributions of bacterial infection in various swabs specimens Collected from Patient**s.**

ISOLATES	FREQUENCY	PERCENT
ALCALIGENES SPP	1	1.1
CITROBACTER SPP	2	2.2
E. COLI	30	32.6
ENTEROBACTER SPP	12	13.0
KLEBSIELLA SPP	18	19.6
PROTEUS SPP	12	13.0
PSEUDOMONAS SPP	15	16.3
PSEUDOMONAS AERUGINOSA	2	2.2
TOTAL	92	100.0

C. Prevalence of different bacterial growth among female and male patients.

A total of 92 sample culture results of patients with suspected infection during the month's period were studied. The isolates and sex distribution of the patients were shown in Table 4. The bacteria isolation rate was in males 48 than in females 44 had a positive bacterial culture result. *E. coli* was the most frequent isolate from female patients.

TABLE IV. Prevalence of different bacterial growth among female and male patients.

ISOLATES	GENDER		TOTAL
	FEMALE	MALE	
ALCALIGENES SPP	**1**	**0**	**1**
CITROBACTER SPP	**0**	**2**	**2**
E. COLI	**16**	**14**	**30**
ENTEROBACTER SPP	**7**	**5**	**12**
PROTEUS SPP	**6**	**6**	**12**
PSEUDOMONAS SPP	**5**	**10**	**15**
KLEBSIELLA SPP	**9**	**9**	**18**
PSEUDOMONAS AERUGINOSA	**0**	**2**	**2**
TOTAL	**44**	**48**	**92**

D. Distribution of clinical specimens.

The swab was the most specimens from which bacteria were isolated, followed by fluid, and the least isolation was from the ear swab specimen.

TABLE V. Distribution of clinical specimens.

SAMPLE	FREQUENCY	PERCENT
BLOOD	8	8.7
EAR SWAB	2	2.2
FLUID	13	14.1
PUS SWAB	69	75.0
TOTAL	92	100.0

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Multidrug-resistant bacterial infections, particularly those caused by Gram-negative pathogens, have appeared as one of the world's greatest health threats. (7) . In this study, the antimicrobial resistance properties of the isolates were analyzed. Almost isolated strains showed a ratio of intermediate to ceftazidime. Another study has also demonstrated in many previous reports that there was a significant positive correlation between an increase in the use of extended ceftazidime and the increased prevalence of ceftazidime-resistant *K. pneumoniae* and *P. aeruginosa*. (10, 11)

TABLE VI. Susceptibility of Gram negative bacteria to ceftazidime

CAZ	FREQUENCY	PERCENT
INTERMEDIATE	79	85.9
RESISTANT	7	7.6
SENSITIVE	6	6.5
TOTAL	92	100.0

E. F. Resistance pattern of isolated pathogens to evaluated antibiotics.

The ceftazidime susceptibility test showed that 30.4% of Escherichia coli isolates were susceptible to ceftazidime. The results of this study indicate that Gram-negative bacteria are less sensitive to ceftazidime. This was in agreement with another study by Karlowsky et al., 6-ceftazidime shows excellent activity in vitro against Enterobacteriaceae.

TABLE VII . Resistance pattern of isolated pathogens to ceftazidime antibiotic.

ISOLATES	CAZ SUSCEPTIBILITY			TOTAL
	I	R	S	
ALCALIGENES SPP	0	1	0	1
CITROBACTER SPP	2	0	0	2
E. COLI	28	0	2	30
ENTEROBACTER SPP	10	1	1	12
PROTEUS SPP	12	0	0	12
PSEUDOMONAS SPP	13	1	1	15
KLEBSIELLA SPP	14	3	1	18
PSEUDOMONAS AERUGLNOSA	0	1	1	2
TOTAL	79	7	6	92

NOTE: R- RESISTANT; S-SENSITIVE; I-INTERMEDIATE.

F. Frequency of isolated pathogens from each nosocomial infection.

E. coli and klebsiella spp were the most prevalent pathogens of pus swab contagion respectively.

TABLE VIII. Frequency of isolated pathogens from each nosocomial infections.

ISOLATES	SAMPLE				TOTAL
	BLOOD	EAR SWAB	FLUID	PUS SWAB	
ALCALIGENES SPP	0	0	0	1	1
CITROBACTER SPP	0	0	0	2	2
E. COLI	2	1	3	24	30
ENTEROBACTER SPP	3	0	4	5	12
PROTEUS SPP	0	0	0	12	12
PSEUDOMONAS SPP	0	1	4	10	15
KLEBSIELLA SPP	3	0	0	15	18
PSEUDOMONAS AERUGINOSA	0	0	2	0	2
TOTAL	8	2	13	69	92

IV. CONCLUSION

The results of this study showed an intermediate susceptibility of Gram-negative bacteria to ceftazidime in vitro. Therefore, ceftazidime alone is not recommended as an experimental treatment.

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The acknowledgements come at the end of an article after the conclusions. Please add here the funding agencies and other acknowledgements

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