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BIOCIDE RESISTANCE AMONG MULTIPLE ANTIBIOTIC RESISTANT LOCALLY ISOLATED URO-PATHOGENIC *E. COLI* ISOLATES

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ABSTRACT

The study aimed to update the resistance profiles of some uro-pathogenic *E. coli* isolates towards some antibiotics and to test the resistance of the multiple resistant isolates towards some commonly used biocides. One hundred *E. coli* isolates were recovered from inpatients and outpatients clinical cases of uncomplicated urinary tract infections. Antibiotic sensitivity against cefotaxime, ciprofloxacin, tetracycline, refampicin, chloramphenicol, gentamicin, imipenim, cefuroxime, ampicillin and erythromycin was determined by agar diffusion. About 20 % of these isolates were found to be multiple antibiotic resistant. On the other hand, about 7% of isolates were completely susceptible to all tested antibiotics. From all isolates, 58% were found to be resistant to at least one antibiotic, 15% were resistant to two antibiotics, where only 1% was found to be resistant to all used antibiotics. Calculated MIC_{50} and MIC_{90} demonstrated that the most effective antibiotic was cefotaxime with MIC_{50}&MIC_{90} of 8 and 32 µg/ml, respectively. MIC values of some biocides (cetrimide, chlorhexidin, chlorocresol and phenylmercuric nitrates) for the multiple resistant isolates were determined and a correlation was found between MIC values of some antibiotics and biocides, namely between chlorocresol and each of chloramphenicol and tetracycline, and between cetrimide and each of gentamicin and tetracycine.

INTRODUCTION

Antimicrobial resistance has been an important problem worldwide. Bacterial resistance to antimicrobial agents has been emerging and rapidly disseminating among many nosocomial and community-acquired pathogens [1]. These organisms have wide variety of antibiotic sensitivity patterns and treatment must be guided by laboratory data [2].Urinary tract infections are very common infections in humans, with *E. coli*, the most common member of the family Enterobacteriaceae, accounting for 75-90 % of all urinary tract infections in both inpatients and out patients [3]. The development of resistance to older antibiotics such as ampicillin, tetracyclines and aminogycosides and the emerging resistance to fluoroquinolone, may substantially limit the antibiotic choices [4].Unlike antibiotics, mechanisms of resistance to non-antibiotic agents, such as preservatives, disinfectants and antiseptics, are less well understood [5]. The frequency of antimicrobial resistance in bacteria has been elevated by increasing usage of antimicrobials. Bacteria have the capacity to adapt rapidly to new environmental conditions and can survive exposure to antimicrobials by using a battery of resistance mechanisms. Some resistance mechanisms are incommon to both biocides and antibiotics [6]. To date, the lack of precise data, in particular on quantities of biocides used, makes it impossible to determine which biocides create the highest risk of generating antibiotic resistance. In the healthcare settings, bacterial resistance to biocides has long been reported with compounds such as: chlorhexidine [7]; quaternary ammonium compounds [8] and chlorocresol [9]. Resistance to cetrimide was previously described [10]. The present study aimed to demonstrate the capability of some multiple resistant uropathogenic isolates to show biocidal resistance and determine the correlation between resistance to some antibiotics and some other biocides.

EXPERIMENTAL

Bacterial isolates

A total of 100 *E. coli* isolates were recovered from urine samples. The samples were collected from inpatients and outpatients of Zagazig University Hospital. Samples were either midstream urine specimens or catheterized urine samples. An aliquot of 0.01 ml of each individual samples were spread on McConkey's agar (Difco, USA) plates, and incubated at 37°C for 24 h. Lactose fermenting colonies were identified as *E. coli* by standard biochemical tests [11].

Methods

Antimicrobial susceptibility testing

Antibiotic susceptibility to nine antibiotics was determined according to National Committee for Clinical Laboratory Standards (NCCLS, 2000) Guidelines [12]. The antibiotic discs used included: gentamicin(10 µg); ciprofloxacin (5 µg); cefotaxime (30 µg), cefuroxime (30 µg), imipenem (10 µg); ampicillin (10 µg); chloramphenicol (30 µg); tetracycline (30 µg) and rifampin (5 µg). The results were interpreted as recommended. *E. coli* ATCC 25922 was used as a control reference strain. Isolates were classified as susceptible (S), intermediate (I), or resistant (R).

Determination of MICs for antibiotics

The MICs of the tested antibiotics were determined by agar dilution method according to NCCLS 2000 B [13]. Two fold serial dilutions of the antibiotic were prepared in Mueller- Hinton agar plates. Control plates (drug free media) were also prepared. Standardized suspensions of the tested organisms were prepared from overnight culture in Mueller-Hinton broth. Plates were spot inoculated by 5µl aliquots (about 10^4 CFU per spot). Results were taken after incubation for 24 hours at 37° C. MIC was taken as the lowest concentration where there was no visible growth.

Determination of MICs for biocides

The same procedures of antibiotics were applied to biocides, except that instead of two fold serial dilution of antibiotic solution, different

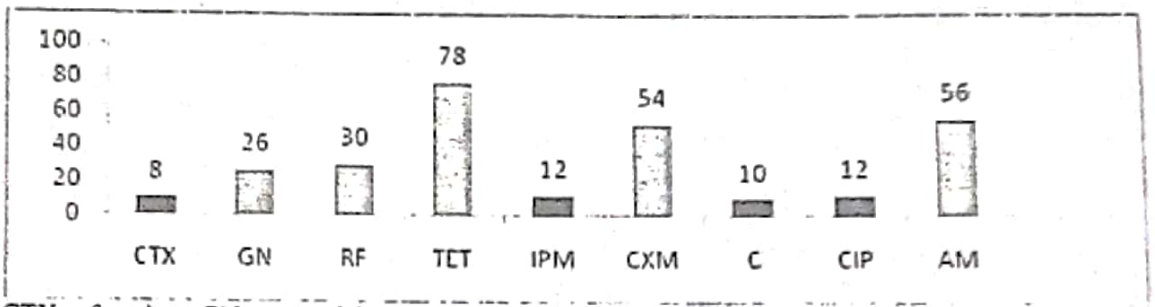
concentrations of biocides were prepared with constant increments of 5000 µg/ ml, 50 µg/ ml, 200 µg/ml and 50 µg/ ml for cetrimide, chlorocresol, chlorhexidine and phenylmercuric nitrate, respectively.

Statistical analysis

All data were subjected to statistical analysis using IBM SPSS statistic base program, and correlation coefficient was calculated according to Pearson rank correlation.

RESULTS

The percentages of resistant isolates to individual antibiotics are demonstrated in figure 1. MICs, MIC_{50}, $MIC_{90}$and MIC range for tested antibiotics and the percentage of resistant isolates for each antibiotic are presented inTable (1).The number and percentage isolates resistant to one or more antibiotics were computed. While only 7% of the isolates were sensitive to all tested antibiotics, 58, 15, 10, 5, 4 and 1% of the isolates were resistant to one, two, three, four, five and six antibiotics, respectively. Twenty of the isolates (20 %) were considered as multiple antibiotic resistant.MICs of six selected antibiotics (Table 2) and four selected biocides namely, cetrimide, chlorhexidine, chlorocresol and phenylmercuric nitrate for the twenty multiresistant isolates are resented in Table (3).Correlation coefficient and significance between antibiotics and biocides MICs were statistically analysed for the multiple resistant isolates and results are presented in Table (4).



CTX:cefotaxime, GN: gentamicin, RF: Rifampin, Do: doxycycline, IPM:imipenem, CXM: cefuroxime, C: chloramphenicol, CIP: ciprofloxacin and AM: Ampicillin

Fig. (1): Percentage of antibiotics resistanturo-pathogenic *E. coli* isolates to selected antibiotics.

Table (1): MIC range, MIC_{50}& MIC_{90} and percentage of isolates resistant to tested antibiotics.

Antibiotics	Resistance Break points* (µg/ ml)	MIC Range (µg/ ml)	MIC_{50} (µg/ ml)	MIC_{90} (µg/ ml)	Percentage of resistant isolates
CTX	≥ 64	0.5- 512	8	32	8
CIP	≥ 4	0.0156-512	0.125	4	12
GN	≥ 8	1- 1024	4	256	26
C	≥ 32	0.5- 1024	2	16	10
TET	≥ 16	1- 1024	32	1024	78
RF	≥ 4	0.5- 128	2	16	30

*according to NCCLS, 2000

Table (2): MICs for different antibiotics for multi resistant isolates

Isolate	MIC in µg/ ml					
	CTX	CIP	GN	C	TET	RF
3	256	0.0156	2	4	1024	16
5	1	0.0156	8	4	1024	32
6	1	0.0156	512	32	512	2
7	512	0.0156	512	256	1024	8
8	1	64	512	256	512	16
10	0.5	0.0156	8	4	512	16
18	1	0.0156	8	4	512	16
21	1	0.0156	1024	1024	1024	16
29	512	512	256	32	1024	16
31	1	0.0156	8	4	1024	16
36	64	64	8	8	64	16
44	1	0.0156	1024	4	1024	16
50	0.5	8	512	1024	1024	16
55	1	8	2	256	1024	16
60	1	0.0625	8	4	1024	16

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Table (2): Continued

Isolate	MIC in µg/ ml					
	CTX	CIP	GN	C	TET	RF
71	1	0.0156	256	16	1024	16
73	1	0.0156	256	64	1024	128
80	1	0.0156	64	64	512	64
81	1	4	32	4	1024	16
97	1	0.0156	8	4	512	16
ATCC 25922	1	0.0156	1	1	4	2

Table (3): MIC values of some biocides for multi resistant isolates**

Isolate	MIC			
	Cet	CC	CHX	PMN
3	0.07	0.015	0.0006	0.0003
5	0.1	0.015	0.0016	0.0003
6	0.1	0.015	0.0006	0.0003
7	0.09	0.015	0.0004	0.0001
8	0.1	0.02	0.0018	0.0001
10	0.06	0.015	0.0008	0.0001
18	0.075	0.02	0.0016	0.0002
21	0.1	0.015	0.0016	0.0002
29	0.1	0.015	0.0008	0.0001
31	0.085	0.015	0.0008	0.0003
36	0.1	0.015	0.0018	0.0003
44	0.08	0.015	0.0008	0.0003
50	0.1	0.02	0.0010	0.0003
55	0.065	0.015	0.0016	0.0001
60	0.055	0.015	0.0010	0.0001
71	0.1	0.015	0.0016	0.0003
73	0.05	0.015	0.0008	0.0001
80	0.1	0.015	0.0008	0.0002
81	0.1	0.015	0.0020	0.0003
97	0.3	0.015	0.0004	0.0003
ATCC 25922	0.0008	0.0005	0.0002	0.00001

**: CC: chlorocresol, PMN: phenylmercuric nitrate, CHX: Chlorohexidine and Cet: cetrimide.

Table (4) the correlation coefficient (r) and significance (p) between MIC values of the tested antibiotics and biocides for multiple resistant isolates.

	CTX r p	CIP r p	GN r p	C r p	Tet r p	Rf r p	Cet r p	CC r p	CHX r p
PMN	-0.28 >0.05	-0.29 >0.05	0.09 >0.05	0.01 >0.05	0.22 >0.05	-0.19 >0.05	0.54 <0.001**	0.18 >0.05	-0.14 >0.05
CHX	-0.21 >0.05	-0.11 >0.05	-0.2 >0.05	-0.03 >0.05	-0.05 >0.05	-0.08 >0.05	0.17 >0.05	0.11 >0.05	--------- -------
CC	-0.03 >0.05	0.03 >0.05	0.2 >0.05	0.32 <0.05*	0.35 <0.01*	0.07 >0.05	0.65 <0.001**	--------- -------	--------- ------
Cet	0.15 >0.05	0.12 >0.05	0.28 <0.05*	0.13 >0.05	0.29 <0.05*	-0.19 >0.05	---------- ---	--------- ----	--------- ------
Rf	-0.15 >0.05	-0.07 >0.05	-0.08 >0.05	-0.08 >0.05	0.16 >0.05	---------- ----	---------- --	--------- -----	--------- -------
Tet	0.28 >0.05	0.13 >0.05	0.24 >0.05	0.24 >0.05	--------- -----	---------- ----	---------- ---	--------- -------	--------- ------
C	-0.05 >0.05	-0.07 >0.05	0.57 <0.001**	---------- ----	--------- -----	---------- -----	---------- ----	--------- ------	--------- ------
GN	0.07 >0.05	0.03 >0.05	---------- ---	---------- --	--------- ----	---------- -----	---------- ----	--------- -------	--------- -------
CIP	0.62 <0.001**	--------- --	---------- -	---------- ---	--------- --------	---------- -----	---------- ------	--------- -------	--------- -------

DISCUSSION

The results revealed high levels of resistance to different antibiotics as tetracycline, rifampicin, and some members of β- lactam group(as ampicillin and cefuroxime).The MIC_{50}& MIC_{90} values and percentage of resistance to tested antibiotics showed that 78% of the tested population was resistant to tetracycline, while only 8% were resistant to cefotaxime. About 7% of tested population was susceptible to all tested antibiotics, 58% was resistant to only one antibiotic and 1% was found to be resistant to all tested antibiotics. Regarding the multiple resistant strains, all isolates were resistant to Tetracycline and 95%, 90%, 45%, 30% and 20% of multiple resistant strains are resistant to rifampicin, gentamicin, chloramphnicol, ciprofloxacin and cefotaxime, respectively.

These results demonstrate that the overall resistance is high. Regarding biocides resistance and according to Russell, 1991[23], MICs resistance break points were: CHX: R≥0.0001 g%, Cet: R≥ 0.0016 g%, PMN: R≥ 0.00005 g% and CC: R≥ 0.012 g%.it was found that all multiple resistant strains were resistant to the tested biocides in this study (chlorocresol, cetrimide, chlorohexidine and phenylmercuric nitrate), revealing a potential correlation between the tested biocide and the antibiotic resistance.

By comparing the magnitude of MIC values for pairs of antimicrobials (biocides and antibiotics), a highly significant correlation between chloramphenicol and gentamicin and between cefotaxime and ciprofloxacin was found. Also a highly significant correlation was found between cetrimide and each of phenylmercuric nitrate and chlorocresol. A significant correlation was found between tetracycline and each of chlorocresol and cetrimide and between gentamicin andcetrimide.

These findings can support the view that the use of active molecules in biocidal products may contribute to the increased occurrence of antibiotic resistant bacteria and vice versa [14,15]. Resistance to both antibiotics and biocides in gram negative organisms is more likely observed as less specific mechanisms, e.g., the outer membrane may act as a nonspecific exclusion of chemically unrelated molecules [16, 17]. There have been some instances where biocides have been claimed to select for resistant gram-negative bacteria. Resistance to CHX, QACs and at least five antibiotics for gram-negative bacteria isolated from urinary tract infections was found and it was proposed that the widespread use of CHX was responsible for selecting antibiotic-resistant strains [23]. There was no evidence of plasmid-linked resistance association [18].

Several publications present the cell target of biocides and the various mechanisms used by the bacterial cell to evade the toxic effect of biocides [19]. It is important to note that antibiotic and biocide antibacterial–actions show many similarities despite some differences in terms of target, killing, behavior and clinical aspects [20]. Among the similarities are (i) the penetration/uptake through bacterial envelope by passive diffusion, (ii) the effect on the membrane integrity and morphology, (iii) the effect on diverse key steps of bacterial metabolism (replication, transcription, translation, transport, various enzymes). Faced with this toxic effect and stress, the response/adaptation of bacterial cells presents some similar defence mechanisms that can overlap the original functions to confer resistance against structurally non-related molecules.Some evidence from bacteriological, biochemical and genetic data does indicate that the use of active molecules in biocidal products may contribute to the increased occurrence of antibiotic resistant bacteria [14, 15].

The selective stress exerted by biocides may favor bacteria expressing resistance mechanisms and their dissemination. Some biocides have the capacity to maintain the presence of mobile genetic elements that carry genes involved in cross-resistance between biocides and antibiotics. The dissemination of these mobile elements, their genetic organization and the formation of biofilms, provide conditions that could create a potential risk of development of cross-resistance between antibiotics and biocides. However, horizontal gene transfer that can be stimulated by external chemical compounds such as biocides are likely triggers of bacterial resistance. Biocides and antibiotics may share some common behavior and properties in their respective activity and in the resistance mechanisms developed by bacteria [21, 22].

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المقاومة للمبيدات الحيوية فى بكتيريا ايشيريشيا كولاى المعزولة من عدوى المجارى البولية ذات المقاومة المتعددة للمضادات الحيوية

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تواجه عدوى المجارى البولية المسببة بواسطة بكتيريا ايشيريشيا كولاى صعوبة فى العلاج بسبب تنامى مقاومة هذه البكتيريا للمضادات والمبيدات الحيوية (المطهرات). تهدف هذه الدراسة لتحديث أنماط المقاومة لبعض هذه العزلات بهدف المساعدة فى علاجها. تم عزل حوالى 100 عترة من بكتيريا ايشيريشيا كولاى من عينات البول للمرضى وتم التعرف عليها بواسطة الاختبارات القياسية. تم قياس حساسية العزلات تجاه بعض المضادات الحيوية ووجد ان حوالى 20% منها تحمل مقاومة متعددة للمضادات, ومنها 1% فقط كانت مقاومة لجميع المضادات الحيوية المستخدمة، بينما وجد أن 7% من العزلات كانت لا تحمل مقاومةلأى من المضادات الحيوية المستخدمة. تم حساب نسبة أقل تركيز مثبط للنمو ولنسبة 50%و 90% من العزلات ووجد ان اكثر المضادات الحيوية تأثيرا على العزلات هو سيفوتاكسيم وأقلها تأثيرا هو مركب تتراسيكلين ، بينما وجد ان باقى المضادات الحيوية المستخدمة كانت فى المجمل ذات تأثير جيد. تم حساب معاملات الارتباط فى المقاومةلثنائيات المضادات الحيوية والمبيدات الحيوية بناء على قيم التركيزات المثبطة للعزلات المقاومة وتبين وجود علاقة ارتباط قوية بين مقاومة البكتيريا لمركب سيتريميد وكل من مركبات فينيل ميركريك نيترات و كلورو كريزول. ايضا علاقة ارتباط قوية بين المقاومة لمضاد الكلورامفنيكول ومضاد الجنتاميسين وبين المقاومة لمضاد سيفوتاكسيم ومضاد سيبروفلوكساسين.