Original paper

Incidence and Diversity of Antimicrobial Resistance Profiles of Enterobacteriacae Bacteria in the Cervico-Vaginal Epithelium of Women in Holy Kerbala Province.

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Abstract

Background: Although vaginal colonization by bacterial species of the enterobcacteriacae family, the significance of their antibiograms and potential role in transmitting of drug resistance is neglected.
Aim of the study: to determine the frequency of occurrence of Enterobacteriacae species and study their antimicrobial susceptibility pattern in cervico-vaginal epithelium of women from kerbala.
Methods: women with vaginal discharges attending Kerbala Teaching Hospital for Gynocology and Obstetric for the period from January to March 2014 were enrolled in this study. High vaginal swabs were processed for the isolation and identification of Enterobacteriacae using standard microbiological techniques. Antimicrobials susceptibility testing was conducted for all of the isolated bacterial species.
Results and discussion: swabs from 100 women were cultured. A total of 101 *Enterobacteriacae* isolates were recovered; 65 *E coli*, 23 *P.mirabilis* and 13 *K.pneumoniae* isolates. All *K.pneumoniae* isolates were resistant to ampicillin, amoxicillin-clavulanic acid and cefotaxime, whereas high resistance rates were detected to those antibiotics among *E. coli* (96.9%, 92.3% and 87.7%, respectively) and *P. mirabilis* (100%, 78.3% and 52.2, respectively). However, all isolates were susceptible to carbapenems (imipenem and meropenem) and low resistance rates detected to quinolones, aminoglycosides, lincosmaide and chloramphenicole. Resistance rates to aztreonam (ATM) were variable, high rates detected among *E coli* (69.2%) and *K pneumoniae* (61.5%) isolates, however, very low rates seen among *P. mirabilis* isolates (4.3%). Furthermore, high resistance rates were reported to tetracyclines and nitrofurantoin among *E. coli* (61.5% and 41.5% respectively), *K. pneumoniae* (69.2% and 100% respectively) and *P. mirabilis* (78.3% and 60.9% respectively). In addition, statistically significant differences were detected in the antibiotics susceptibility testing among the types of isolates to cefotaxime ($p= 0.001$), aztreonam ($p= 0.000$), cholramphenicole ($p= 0.000$), ciprofloxacin ($p= 0.046$), nitrofurantoin ($p= 0.002$) and amikacin ($p= 0.007$).
Conclusions: Enterobacteriacae colonizing the cervico-vaginal epithelium are resistant to several important anitbiotics (multiple drug resistant) and thus may pose significant threat in transmission of drug resistant bacteria.
Keyword: Enterobactriacae, antibiotic susceptibility testing, high vaginal swabs.

Introduction

Vaginal colonization by drug resistant bacteria may represent a neglected source for the dissemination of those dangerous bacteria [1, 2]. In addition, during pregnancy, due to the risk of the adverse effects of antibiotics on the

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embryos and embryogenesis, it is not a practical to eliminate vaginal colonization by resistant bacterial species.

Pathogens resistant to multiple antibiotics are rapidly emerging, entailing important consequences for human health. The heightened use/misuse of antibiotics in human medicine, agriculture and veterinary is primarily contributing to the phenomenon [2].

Bacteria have developed several strategies confront the effects of antimicrobial agents, and have evolved highly efficient means for clonal spread and for the dissemination of resistance traits [3]. Extended-spectrum β-lactamases (ESBLs) are capable of hydrolyzing broad spectrum cephalosporins and monobactams. In addition, ESBL producing organisms exhibit co-resistance to many other classes of antibiotics resulting in limitation of therapeutic options. The resistant organisms are now a worldwide problem [4, 5] and their incidence is being continuously increasing with limited treatment alternatives. It becomes necessary to know the prevalence of these organisms and to formulate treatment policy [4]. Significant increases have been observed in the prevalence of MDR Gram-negative bacteria (GNB) [6] highlighted by reports that as high as 53% of GNB are co-resistant to aminoglycosides, third generation cephalosporins and quinolones [6, 7]. The high prevalence of resistance to quinolones and third-generation cephalosporins is alarming because these antimicrobials have broad spectrum activity and are often used empirically [8]. Colonization with MDR-GNB is a risk factor for subsequent infection and these organisms have been shown to colonize more persistently and to co-colonize often with different species of MDR-GNB [9].

Vaginal colonization by members of *Enterobacteriacae* bacteria represents a real threat specially to neonates, however, few information is available regarding their antibiotic resistance, therefore; this study was carried out to detect incidence and diversity of to determine the frequency of occurrence of Enterobacteriacae species and study their antimicrobial susceptibility pattern in cervico-vaginal epithelium of women from kerbala .

Materials and Methods

Sampling and samples processing

Women with vaginal discharges attending Kerbala Teaching Hospital for Gynocology and Obstetric for the period from January to March 2014 were enrolled in this study.

High vaginal swabs were collected using sterile cotton swab (Himedia, Mumbai) by a nurse under the supervision of the attending Gynaecologist. The swabs were immediately placed into Stuart's transport media (Himedia, Mumbai) and transported to the laboratory at room temperature within 5-6 hours. The swabs, then, were processed for the isolation and identification of Enterobacteriacae using standard microbiological techniques.

Susceptibility Testing

Disk-diffusion tests were carried out with antibiotic-containing disks (Bioanalyse) on Mueller-Hinton agar plate (Himedia). The results were expressed as susceptible or resistant according to the criteria recommended by the Clinical Laboratory Standards Institute (CLSI) (10). The following antimicrobial agents were tested: ampicillin (AMP: 10ug), amoxicillin- clavulanic acid (AMC: 20/10ug), cefotaxime (CTX: 30 µg), aztreonam (ATM: 30 µg), imipenem (IMP: 10 µg), meropenem (MEM: 10 µg), clindamycin (CN: 10 µg),
chloramphenicole (C: 30 µg), nalidixic acid (NA: 30 µg), ciprofloxacin (CIP: 5 µg), levofloxacin (LEV: 5 µg), tetracyclin (TE: 30 µg), nitrofurantoin (F: 300 µg) and amikacin (AK: 30 µg).

Statistical Analysis

The Chi Square test was used for statistical comparison of groups; values < 0.05 were regarded as significant [11].

Results

A total of 101 *Enterobacteriacae* isolates were recovered in this study. *E coli* was the most prevalent where the number of isolates was 65, followed by *P.mirabilis* with 23 isolates, whereas the least one was *K.pneumoniae* with 13 isolates (table 1).

Table 1. Type of isolates and their frequencies

Isolates	number	Frequency out of the total number of isolates(%)
Escherichia coli	65	64.4%
K.pneumoniae	13	12.8%
P.mirabilis	23	22.8%
Total	101	

Table 2 shows the antibiotics susceptibility testing of the isolates to different antibiotics. High rates of resistance were detected to ampicillin, where all isolates of *P.mirabilis* and *K.pneumoniae* in addition to 63 out of the 65 (96.9%) *E coli* isolates were resistant. The resistance rates to ampicillin-clavulanic acid were also high; 60/65 (92.3%) of *E. coli* isolates, and all *K.pneumoniae* (100%) and 18/23 (78.3%) of the *P.mirabilis* were resistant. High resistance rates were also detected to cefotaxime; 57/65 (87.7%) of the *E coli* and all *K.pneumoniae* (100%) were resistant, whereas less resistance rate (12/23, 52.2%) was detected among *P.mirabilis* isolates.

Both *E. coli* and *K .pneumoniae* isolates demonstrated high resistance rates to aztreonam (ATM) (, 69.2% and 61.5%, respectively) and, in contrast, low resistance rate was detected among *P. mirablis* isolates (4.3%).

All of the isolates in this study were susceptible to carbapenems (e.g. imipenem, meropenem). This indicates that none of the isolates can produce metallo-beta-lactamases.

No significant resistance to aminoglycosides could be detected among the isolates in this study as all of the *P mirabilis* and *K.pneumoniae* isolates, as well as 64/65 *E coli* isolates were susceptible or intermediately susceptible to amikacin. Only one *E coli* isolate was found to be resistant to amikacin.

The incidence of resistance to fluoroquinolone (to ciprofloxacin, levofloxacin) and quinolones were variable. The resistance rates to fluoroquinolone were very low; all *P.mirablis* strains were susceptible and only one *E coli* isolate and one *K pneumoniae* isolate were resistant to ciprofloxacin. In addition, all *P.mirabilis* and *K.pneumoniae* were susceptible to levofloxacin, whereas among 65 *E coli* isolates, only 2 isolates were resistant to this antibiotic. On the other hand, a avaraible rates of resistance rates were detected to the Nalidixic acid; resistance was seen in 11 (16.9%) *E. coli* and 2 (15.4%) *K.pneumoniae*, whereas, all *P.mirabilis* were susceptible or intermediately-susceptible to nalidixic acid.

A relatively high incidence of resistance were seen to nitrofurantoin; all *K.pneumoniae* isolates were resistant to this antibiotic, whereas, 27 (41.5%), of *E coli* and 14 (60.9%) of *P.mirablis* isolates were resistant.

All isolates belong to *K.pneumoniae* and *P.mirabilis* were susceptible or moderately-susceptible to chloram-phenicol, whereas, 5 isolates (7.7%) belong to *E.coli* were resistant to this antibiotic.

Majority of the isolates in this study were resistant to tetracycline; 40 (61.5%) of *E. coli*, 9 (69.2%) of *K.pneumoniae*, and 18 (78.3%) of the *P.mirabilis* isolates.

While the majority of *E coli* and *K.pneumoniae* isolates were susceptible to clindamycin (n= 52, 80.0% and 12, 92.3%, respectively), a substantial number of the *P. mirabilis* isolates were resistant to this antibiotic (n=9, 39.1%)

Statistically significant differences were detected in the antibiotics susceptibility testing among the types of isolates to cefitriaxone (*p= 0.001*), aztreonam (*p=0.000*), cholramphenicole (*p=0.000*), ciprofloxacin (*p=0.046*), nitrofurantoin (*p= 0.002*) and amikacin (*p= 0.007*).

Table 2. Antibiotic susceptibility testing *E. coli, K. pneumoniae* and *P.mirabilis*

Class of Antibiotic	Antibiotic tested	E coli (n= 65)			K pneumonia (n=13)			P. mirabilis (n=23)			Chi-square (p-value)
		S	I	R	S	I	R	S	I	R	
Beta lactam antibiotics	AMP10ug	1	1	63	0	0	13	0	0	23	0.889
	AMC10ug	2	3	60	0	0	13	0	5	18	0.058
	CTX30ug	7	1	57	0	0	13	7	4	12	0.001
	ATM30ug	18	2	45	0	5	8	22	0	1	0.000
	MEM10ug	64	1	0	13	0	0	23	0	0	0.756
	IPM10ug	65	0	0	13	0	0	23	0	0	a
lincosamides	CN10ug	54	2	9	12	0	1	14	0	9	0.055
Chloramphenicole	C30ug	55	5	5	13	0	0	11	12	0	0.000
Fluoroquinolone and quinolone antibiotics	NA30ug	48	6	11	8	3	2	20	3	0	0.171
	CIP5ug	64	0	1	11	1	1	23	0	0	0.046
	LEV5ug	63	0	2	13	0	0	23	0	0	0.568
Tetracyclines	TE30ug	24	1	40	4	0	9	5	0	18	0.644
Nitrofurantoin	F300ug	33	5	27	0	0	13	9	0	14	0.002
Aminoglycosides	AK30ug	61	3	1	9	4	0	23	0	0	0.007

a. No statistics are computed because IPM10ug is a constant

Discussion

This study was designed to determine the possible incidence and diversity of antimicrobial resistance profiles of *Enterobacteriacae* bacteria colonzing the cervico-vaginal epithelium of women attending a main gynecology and obstetrics hospital in Holy Kerbala Province, Iraq.

In this study, three species of *Enterobacteriacae* were isolated; *E coli* were the most prevalent species. *E. coli* is considered by many researchers as a normal inhabitant of the vagina and is seen to colonize upto 20% of pregnant women [12-14]. These colonizing isolates can sometimes cause complications during pregnancy or can be transmitted to the neonate leading to neonatal infection [1, 12, 15, 16]. Ability to colonize and cause infections has been attributed to the presence of several virulence genes in these isolates [14, 16].Vaginal colonization with E. coli is reported as a risk for very low birth weight delivery and other perinatal complications [12].

In this study, *P.mirablis* were isolated from 23 women. This microorganism was also reported to colonize the vagina in other studies. During a systematic survey, maternal carriage of Proteus mirabilis was found over a 25-day period in 18 pregnant women admitted to the delivery ward in a French maternity hospital. Five neonates born to these mothers were found to be colonized with *P. mirabilis* [17]. Neonatal meningitis caused by *P. mirabilis* is often accompanied by brain abscesses or ventriculitis and carries high mortality and morbidity rates [18].

In the current study, *K .pneumoniae* were isolated from 18 women. This bacterium is not uncommon colonizer of the vagina as it frequently reported to colonize vagina and, sometimes, cause bad consequences [19].

High rates of resistance were detected to ampicillin among all of the three species.

The resistance rates to ampicillin-clavulanic acid were also high. Resistance to ampicillin-clavulanic acid indicates that the isolates may harbor types of extended-spectrum β-lactamases (ESBLs) that is not inhibited by lactamases inhibitor (clavulanic acid). High resistance rates were also detected to cefotaxime. Collectively, resistance to AMC and CTX indicate the presence of CTX-M beta lactamases. Isolates that produce CTX-M typically are resistant to cefotaxime and some may have reduced susceptibility to inhibitors of beta lactamases.

Both *E. coli* and *K .pneumoniae* isolates demonstrated high resistance rates to aztreonam. Resistance to aztreonam could be mediated by K1 beta lactamase. Hyper-producers of the K1 enzyme also are resistant to ampicillin and other penicillins, cefuroxime, and ceftriaxone, but are susceptible to ceftazidime. Aztreonam is the only available β-lactam that is inherently impervious to metallo-β-lactamases, thus it would theoretically present an attractive option for the treatment of infections with pathogens that produce these enzymes. Unfortunately, in most cases, these organisms come with an onslaught of other β-lactamases (i.e., CTX-M type, CMY type, etc.) that readily hydrolyze aztreonam [20].

Studies from worldwide have reported isolation of drug resistant *E. coli* among vaginal isolates of pregnant women [13, 21, 22]. Transmission of these resistant strains to the neonate can prove fatal in whom early detection is challenging and treatment options are limited. Outbreaks in neonatal wards and adverse outcome due to drug resistant E. coli infection have been reported [23, 24]. Thus identification and elimination of these resistant strains at the maternal level can have an impact on the reduction of fatal outcome in neonates especially in developing countries where the neonatal mortality rate is high [25].

All of the isolates in this study were susceptible to carbapenems (e.g. imipenem, meropenem). This indicates that none of the isolates can produce metallo-beta-lactamases.

No significant resistance to aminoglycosides could be detected among the isolates in this study.

The incidence of resistance to to ciprofloxacin and levofloxacin were variable to nalidixic acid. The first quinolone, nalidixic acid (possessing a naphthyridone core), was introduced into clinical use in 1962 [26]. In the mid-1980s, ciprofloxacin, a fluoro-quinolone (with a quinolone core) that had a wider spectrum of in vitro antibacterial activity, particularly against gram-negative bacteria, first became available clinically [27]. Since then, newer agents with increased antimicrobial activity against gram-positive pathogens have been developed, but the activity of ciprofloxacin against gram-negative pathogens has been largely unsurpassed [28]. The main mechanism of quinolone resistance is the accumulation of mutations in the bacterial enzymes targeted by fluoroquinolones: DNA gyrase and DNA topoisomerase IV [29].

A relatively high incidence of resistance were seen to nitrofurantoin.

Regarding resistance to clindamycin, *K. pneumoniae* showed less resistance rate in comparison to *P.mirabilis*. Clindamycin is a member of lincosamides family of antibiotics. It inhibits protein synthesis by attaching to the 50S ribosomal subunit causing termination of the growing protein chain. Resistance to macrolides such as erythromycin, and lincosamides such as clindamycin, usually is due to an e*rm* gene. These *erm* genes code for production of an RNA methylase enzyme that modifies the ribosomal binding site of macrolides, lincosamides, and streptogramins B. This is known as MLS resistance. Strains possessing *ermA, ermB* or *ermC* typically are erythromycin resistant but, when initially tested, may appear clindamycin susceptible (especially *ermC* strains). In such isolates clindamycin

resistance is expressed after induction with erythromycin.

All isolates belong to *K.pneumoniae* and *P.mirabilis* and majority of *E. coli* isolates were susceptible or moderately-susceptible to chloram-phenicol.

Majority of the isolates in this study were resistant to tetracycline. Tetracyclines (e.g. tetracycline, mino-cycline and doxycycline) bind to the 30S subunit of the ribosome and block the attachment of transfer RNA (tRNA). Since new amino acids cannot be added to the growing protein chain, synthesis of protein is inhibited. The action of tetracyclines is bacteriostatic.

Statistically significant differences were detected in the antibiotics susceptibility testing among the types of isolates to cefitriaxone (*p= 0.001*), aztreonam (*p=0.000*), cholramphenicole (*p=0.000*), ciprofloxacin (*p=0.046*), nitrofurantoin (*p= 0.002*) and amikacin (*p= 0.007*).

In conclusions, members of the Enterobacteriacae colonizing the cervico-vaginal epithelium are resistant to several important anitbiotics (multiple drug resistant), especially several generations of the β-lacatm antibiotics and antibiotics of other classes such as nitrofurantoin and tetracyclins. Thus, those bacterial species may pose significant threat in transmission of drug resistant bacteria.

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