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Multi-drug resistance profile of extended spectrum β-Lactamases producing Escherichia coli isolated from sheep in Mosul city

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Abstract

Multi-drug resistance (MDR) *Escherichia coli* have become a major threat due to their ability to overcome different types of antibiotics. However, Extended Spectrum β-lactamase *E. coli* (ESBLE) imposes an additional threat due to their ability to resist the 3rd generation cephalosporins. Accordingly, our study aimed to investigate the antibiogram profile of ESBLE isolates obtained from sheep. A Total of 40 ESBLE isolates were included in this study which represents sheep fecal and milk samples. Twelve antibiotics were selected to perform antibiotic sensitivity tests following standard microbiological methods. The results of the study showed that the highest resistance percentages were recorded for tetracycline 97.5%, ciprofloxacin 80%, trimethoprim/sulfamethoxazole 65%, and streptomycin 57.5%. While other antibiotics recorded lesser values. On the other hand, all isolates were susceptible to gentamycin and tobramycin each at 92.5%, followed by chloramphenicol and levofloxacin each at 82.5% and nitrofurantoin 72.5%. While fewer values of sensitivity were recorded for streptomycin, trimethoprim /sulfamethoxazole, azithromycin, nalidixic acid, ciprofloxacin and, tetracycline. The study concluded that ESBLE of sheep origin that have additional resistance features to other antibiotics could be a major threat for spreading resistance and contaminating the environment and finally impose negative impact for response to antibiotic treatment in humans.

Introduction

Escherichia coli is one of the most important Enterobacteriaceae members that is capable of causing a variety of clinical infections in different species of animals and also humans (1,2). During the last decade, the scene was become more complicated due to the involvement of extended-spectrum ß-lactamases (ESBLs) type *E. coli* in many animals infections including man (3,4). Recently, the presence of such type of bacterial resistance has been reported in Iraq, in poultry (5), bovine milk (6), and feces of shepherd dogs (7). Furthermore, in a recent study on sheep, we were successfully able to isolate ESBL *E. coli* (ESBLE) from milk and feces of both diseased and healthy ewes in addition to the feces of their lambs (data submitted

for publication). Such a wide distribution arise an important question about the possibility of circulating of such resistant bacteria between animals, the environment, and humans, and this challenging the legislation that has been regulated by the international organizations such as WHO, FAO, and OIE in terms of One Health concept to mitigate the effect of antibiotic resistance (8,9). On the other hand, the presence of multidrug resistance *E. coli* has been reported worldwide which includes resistance to different groups of antibiotics such as aminoglycosides, fluoroquinolones, phenicols, macrolides, trimethoprim /sulfonamides, tetracyclines, nitrofuran derivatives, and carbapenems (4,10). Such type of resistance surely increases the concern about successive treatment failure of current antibiotics and compels the drug companies to find

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other solutions to overcome this obstacle (4,11). In Iraq, as one of the developing countries, the arbitrary and misuse of different types of antibiotics beyond the therapeutic and recommended doses in both human and veterinary sectors has complicated the problem, especially in the absence of clear and strict regulations that limited the use of antibiotics. Nevertheless, sheep rising in Iraq, as the main livestock trading, has a big share of such antibiotic misuse (12). Therefore, we believe that sheep that shedding ESBLE that could have additional features of resistance to other antibiotics might exaggerate the problem and accelerate the resistance range to the extent that develops multidrug resistance bacteria (10,13). Accordingly, our study aimed to investigate the antibiogram pattern of ESBLE that have been isolated from sheep.

Materials and methods

Bacterial isolates

In this study, 40 positive ESBLE isolates were selected to perform an antibiotic susceptibility test. The obtained isolates were recovered from our previous study targeting detection of ESBLE from sheep in Mosul city. The details of ESBLE are listed in (Table 1). These isolates were previously confirmed for their ability to produce β-lactamases by culture on MacConkey agar plus cefotaxime, as third-generation cephalosporin according to Ahmed (6). Also, these isolates were tested by polymerase chain reaction (PCR) and confirmed positive for their ability to produce at least one of the resistance genes including CTX-M, SHV, and TEM genes (data submitted for publication).

Table 1: Number of ESBLE isolates used in the study

Sample type	Tested sample (n)
Milk (clinically healthy ewes)	7
Feces (clinically healthy ewes)	13
Feces (clinically healthy lambs)	12
Milk (ewes with clinical mastitis)	4
Feces (ewes with clinical mastitis)	4
Total	40

Antibiotic susceptibility test

Antibiotic susceptibility test was performed according to Kirby-Bauer disc diffusion method following Clinical and Laboratory Standards Institute (CLSI) (14). The antibiotic discs (Bioanalyse/ Turkey) used in this study include, gentamycin 10 µg, streptomycin 25 µg, tobramycin 10µg, nalidixic acid 30 µg, ciprofloxacin 10 µg, levofloxacin 5 µg, chloramphenicol 10 µg, azithromycin 15 µg, trimethoprim/sulfamethoxazole 1.25/23.75 µg, tetracycline 10 µg, nitrofurantoin 100 µg, imipenem 10 µg. Briefly, bacterial suspension (equivalent to 0.5 McFarland opacity standard) was prepared for each isolate and inoculated on Mueller-Hinton agar plates using a sterile swab. Then, the discs were placed on plates and incubated

at 37ºC for 24 h. The zone of inhibition of each disc was measured using a digital caliper (Ingco, China). The results were interpreted by comparing inhibition zone diameters according to manufacturer instructions.

Results

The antimicrobial resistance pattern of the ESBLE showed an alarmingly high resistance to tetracycline 97.5%, ciprofloxacin 80%, trimethoprim/sulfamethoxazole 65%, and streptomycin 57.5% followed by azithromycin 52.5%. While nitrofurantoin, nalidixic acid, chloramphenicol, levofloxacin, and gentamycin recorded fewer values. On the other hand, all isolates were susceptible to gentamycin and tobramycin each at 92.5%, followed by chloramphenicol and levofloxacin each at 82.5% and nitrofurantoin 72.5%. While fewer rates of sensitivity were recorded for streptomycin, trimethoprim /sulfamethoxazole, azithromycin, nalidixic acid, ciprofloxacin, and tetracycline. Imipenem was the only drug that was 100% sensitive for all ESBLs producers (Table 2). Additionally, antibiotic resistance was different according to the source of samples, the feces and milk of ewes with clinical mastitis showed 4/4 (100%) resistance for each streptomycin, ciprofloxacin, tetracycline, and trimethoprim/ sulfamethoxazole. While feces and milk of healthy ewes showed 13/13 (100%) resistance to tetracycline and fewer values were recorded for other antibiotics.

Table 2: Antimicrobial resistance profiles among ESBLE isolates from sheep

Antibiotic	Number (%)		
	Resistance	Intermediate	Sensitive
Gentamycin	3 (7.5)	0	37 (92.5)
Streptomycin	23 (57.5)	0	17 (42.5)
Tobramycin	0	3 (7.5)	37 (92.5)
Nalidixic acid	8 (20)	22 (55)	10 (25)
Ciprofloxacin	32 (80)	0	8 (20)
Levofloxacin	6 (15)	1 (2.5)	33 (82.5)
Chloramphenicol	7 (17.5)	0	33 (82.5)
Azithromycin	21 (52.5)	8 (20)	11 (27.5)
SXT	26 (65)	0	14 (35)
Tetracycline	39 (97.5)	0	1 (2.5)
Nitrofurantoin	11 (27.5)	0	29 (72.5)
Imipenem	0	0	40 (100)

Discussion

Human and veterinary medicine share many critical classes of antibiotics especially the 3rd and 4th generation cephalosporins (10). However, the arbitrary prescription and misuse of antibiotics even as prophylactic or as growth promoters have complicated the problem of antibiotic resistance which emerged new strains of bacteria that have

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been listed under MDR (13-16). *E. coli* is one of the most important commensals in both humans and animals with worldwide distribution (17,18). Nevertheless, the emerging of this bacterium as the main contributor of ESBL leads to the transmission of resistance to other bacterial species (1,17). Furthermore, resistance to other antibiotic classes means that we are facing a new problem that has a negative impact on human public health due to the substitution of sensitive normal flora with those with MDR properties (19,20). Our result showed that tetracycline, trimethoprim/ sulfamethoxazole, and streptomycin were recorded the highest resistance among the tested antibiotics. This was expected because these broad-spectrum antibiotics are commonly used in veterinary clinics for the treatment of bacterial infections caused by both Gram-positive and Gram-negative bacteria. These results were in agreement with the recent study in Malaysia by Haulisah *et al.* (10) as they recorded complete or almost complete resistance 100% against each amoxicillin and erythromycin, tetracycline 95.1% and more than 90% of isolates were not-susceptible to seven of nine tested antibiotics. Another recent study by Pehlivanoglu *et al.* (4) also reported that the highest resistance percentage of *E. coli* was found against tetracycline and streptomycin 52.9% from dairy cattle and sheep farms in Burdur, Turkey. While Batabyal *et al.* (21) found that amoxicillin/clavulanic acid 83.3%, tetracycline 75 %, and gentamycin 58.3% were the most antibiotic resistant ESBLE from milk samples collected from healthy cows in different parts of West Bengal, India. Feces of healthy ewes and lambs showed high resistance to tetracycline and ciprofloxacin and this could explain the possibility of transmission of resistance from ewes to lambs and may also contribute to disseminate resistance to the environment. This was in agreement with previous studies (4,12). It is clear that ESBLE keeps developing its antibiotics resistance ability and generates a new type of MDR bacteria that have a wide range of resistance to various antibiotics.

Conclusion

The involvement of MDR type of ESBLE in sheep feces and milk could impose an additional burden and left few choices to win the battle against antibiotic resistance.

Acknowledgments

Conflict of interest

The authors declare that they have no conflict of interest.

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صورة المقاومة المتعددة للأدوية لجراثيم الإيشيريكيا القولونية المنتجة لأنزيمات البيتا لاكتام واسعة الطيف والمعزولة من الضأن في مدينة الموصل

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الخلاصة

تعتبر الإيشريكيا القولونية ذات صفة المقاومة المتعددة للأدوية خطرا كبيراً وذلك بسبب قدرتها على تجاوز العديد من المضادات الحيوية. ومع ذلك تفرض الإيشريكيا القولونية المنتجة لأنزيمات البيتا لاكتام الواسعة الطيف مخاوف إضافية وذلك لقدرتها على مقاومة السيفالوسبورينات من الجيل الثالث. وفقاً لذلك فقد هدفت دراستنا إلى التحري عن صفة المقاومة للمضادات الحيوية لجراثيم الإيشريكيا القولونية المنتجة لأنزيمات البيتا لاكتام الواسعة الطيف المعزولة من الضأن. تضمنت الدراسة ٤٠ عزلة جرثومية من جراثيم الإيشريكيا القولونية المنتجة لأنزيمات البيتا لاكتام الواسعة الطيف والتي تمثل عينات مأخوذة من براز وحليب الضأن. تم اختيار ١٢ مضاد حيوي لإجراء فحص الحساسية للمضادات الحيوية باستخدام طرق الأحياء المجهرية القياسية. أظهرت الدراسة أن اعلى نسبة مقاومة كانت للمضاد الحيوي التتراسايكلين ٩٧,٥%، والسايبروفلوكساسين ٨٠%، والترايميثبريم/السلفاميثاكسازول ٦٥%، والستربتومايسين ٥٧,٥%، في حين سجلت المضادات الحيوية الأخرى قيما اقل للمقاومة. في الجانب الأخر كانت اغلب العزلات حساسة للجنتامايسين والتوبرامايسين وبنسبة ٩٢,٥% لكل منهما، ثم الكلورامفينكول والليفوفلوكساسين بنسبة ٨٢,٥% لكل منهما والنايتروفيورانتين بنسبة ٧٢,٥%، في حين سجلت نسب مئوية اقل للحساسية لكل من الستربتومايسين والترايميثبريم /السلفاميثاكسازول والأزثرومايسين وحامض النالدكسك والسايبروفلوكساسين والتتراسايكلين. خلصت الدراسة أن وجود الإيشريكيا القولونية المنتجة لأنزيمات البيتا لاكتام الواسعة الطيف في الضأن والتي تمتلك خصائص إضافية لمقاومة المضادات الحيوية الأخرى قد يشكل خطرا كبيرا لانتشار المقاومة وتلوث البيئة وبالتالي قد ينعكس سلباً على الاستجابة للعلاج باستخدام المضادات الحيوية في الإنسان.