ACTIVITY OF BACTERIAL ANTIBIOTICS AGAINST SOME PATHOGENIC BACTERIA ISOLATED FROM CALVES DIARRHOEA IN BAGHDAD (part I)

R. N. Abdulridha O. M.S. Ibrahim
Dept. of Physiology &Pharmacology - College of Vet. Med. University of Baghdad
Oroobam2000@gmail.com

ABSTRACT

This study was aimed to isolate, identify and characterize the bacterial pathogens causing calf diarrhea in Baghdad province and study it's susceptibility toward different antibiotics. The study was conducted on 105 faecal samples collected directly from the rectum of diarrhoeic calves and brought to the laboratory for bacteriological examination. Isolation and identification of the microorganisms were confirmed on the basis of their, staining, cultural, morphology and biochemical tests. Furthermore, antibacterial test was study for different clinical isolates of pathogenic bacteria toward varying concentration of antibiotics by disc diffusion technique. samples were examined for the isolation of bacteria of which 45 (42.85%) samples were positive for *E. coli,* 22 (20.95%) samples were positive for *Salmonella spp,* 16 (15.23%) samples were positive for *Staphylococcus,* 12 (10.5%) samples were positive for Mixed infection, 10 (9.52%) samples were negative for bacteria. The susceptibility study revealed that most of the *E. coli, Salmonella spp.*, and *Staphylococcus spp*. were resistant to ampicillin, amoxicillin, erythromycin, gentamicin, tetracycline, streptomycin and trimethoprim / sulfamethoxazole with varying percentages, and susceptible to ciprofloxacin and azithromycin. The findings of the present study indicate that the clinical isolates of pathogenic bacteria resistance to a number of bacterial antibiotics.

Key words: *E.coli, salmonella, staphylococcus,* Calf; Diarrhea.

مجلة العلوم الزراعية العراقية –2018 :49(5):847-854 عبد الرضا وابراهيم

فعالية المضادات الحيوية البكتيرية ضد بعض البكتريا المرضية المعزولة من اسهال العجول في بغداد

ريهام نجم عبد الرضا عروبة محمد سعيد إبراهيم
باحث أستاذ
فرع الفسلجة والأدوية – كلية الطب البيطري–جامعة بغداد

المستخلص

أجريت هذه الدراسة لعزل وتوصيف وتحديد مسببات الأمراض البكتيرية المسببة لإسهال العجول في محافظة بغداد. أجريت الدراسة على 105 عينة من البراز تم جمعها مباشرة من مستقيم العجول المصابة بداء الإسهال وأحضرت إلى المختبر لعزل وتشخيص البكتيريا. تم تأكيد عزل وتوصيف الجراثيم على اساس الاختبارات الصبغية والشكلية والكيموحيوية. علاوة على ذلك، تمت دراسة فعالية عدد من المضادات الحيوية البكتيرية وبتراكيز مختلفة من خلال تقنية الاقراص في الأكار. أظهرت نتائج العزل لعينات البراز تلك أن 45 عينة (42.85٪) تحتوي جرثومة الايشريكيا القولونية، 22 عينة (20.95٪) جرثومة السالمونيلا، 16 عينة (15.23٪) جرثومة المكورات العنقودية، 12 عينة (10.5٪) لجراثيم مشتركة و 10 عينات (9.52٪) سالبة للبكتيريا. أظهر فحص الحساسية لجراثيم الايشريكيا القولونية والسالمونيلا والمكورات العنقودية مقاومة للأمبسلين والأموكسيسيلين وإريثروميسين والجنتاميسين والستربتومايسين والتتراسايكلين وتريميثوبريم / سلفاميثوكسازول وبنسب متباينة. وحساسة فقط للسيبروفلوكساسين والأزيثروميسين. تشير نتائج الدراسة الحالية إلى أن معظم الجراثيم المرضية المعزولة مقاومة للمضادات الحيوية البكتيرية.

كلمات مفتاحية: الأشريكيا القولونية، السالمونيلا، المكورات العنقودية، العجل، اسهال.

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INTRODUCTION

Calf diarrhea caused by bacterial infection has a bad effect on the dairy industry all over the world when calves are reared intensively, It involves significant economic loss for labor and capital, calf mortality, loss in calf value and veterinary costs (13,33). The incidence of diarrhea in calves under 30 days of age varies between 10 % and 20%. Overfeeding, overpopulation, cold temperature, bad hygiene, artificial feeding and colostrums deprivation are all predisposing factor which can be important in the complex etiology of the disease (6). Diarrhoea caused by different enteropathogens has been recognized as a major clinical problem for calves worldwide. Among these bacteria *Eschirechia coli* as "white scour", *Salmonella typhyimurium, Clostridium perfringens* and *Staphylococcus aureus* are believed to be the major microbial causes of diarrhea in calves (1,10). Farm geographic location, management practices, as well as herd size affect considerably the prevalence of the pathogen (9). Antimicrobial agents are considered popular to fight diarrhea in calves. Nevertheless, their wide spectrum of activity, the emergence of microbial tolerance of different antimicrobial agents has become a well-known phenomenon, which represents a major concern (18). The frequent use of antibacterial agents has created the selective pressure to enhance the rising rates in antibiotic tolerance to different types of bacteria (15,24). Consequently, due to the truth that microorganisms developed resistance against several types of antimicrobial agents, communicable diseases persist to be one of the most important public health problems in different countries. In addition, the disadvantages of frequently used antimicrobial agents are not only the development of multiple drug resistance, but also adverse side effects (21). As a result of antimicrobial resistance in the diarrhea of pre-weaned calves (18,33). therefore, important that sensitivity of different bacteria isolated from diarrhoeic calves needs to be studied from time to time in order to formulate appropriate therapeutic measures. Therefore, the objectives of the current study were thus to isolate and identify various bacteria from feces of calves suffering from diarrhea in Baghdad, and evaluate it's antimicrobial activity.

MATERIALS AND METHODS

Selection of study area:

The research study was carried out in calf farm, samples were obtained from different locations (Nahrawan, Dujail, Fdhiliya, Abu-Ghraib) during the period of October 2017 to February 2018

Samples Collection

One hundred and five sample from diarrhoec calves collected into sterile plastic tubes and submitted to the laboratory of the Department of physiology and pharmacology. College of veterinary medicine university of Baghdad. Isolation and identification of bacterial pathogens were performed as per procedures described by (26,29).

Isolation and identification of bacterial pathogens from calf diarrhoea

Cultural characteristics: Cultural characteristics were studied depending on colony morphology (color, size, consistency, density), Primary culture was performed in Nutrient agar and Nutrient broth media. Subcultures were performed in MacConkey agar, Blood agar, Staphyloccous Agar No.110, Eosin-Methylene Blue agar, Salmonella-Shigella agar, Manitol salt agar, and Simmon's citrate agar

Morphological characterization

The representative bacterial pathogens were isolated from suspected cases of fecal samples then stained with Gram's staining techniques (26).

Biochemical characterization

Isolated bacterial was identified by studying morphological and some biochemical characteristics test**:** Indole, Catalase, Methyl-Red, Voges–Proskauer, Citrate utilization, MIU, Triple sugar iron and Hydrogen sulphide

Maintenance of stock culture

The stock culture was maintained following the procedures of (11). Pure culture of the isolated organisms were stored in sterilized 80% glycerin and used as stock culture.

In-vitro Antibacterial Activity of standard antimicrobial: Commercially available discs (9 mm diameter) preloaded with ampicillin (30 µg/disk), amoxicillin (20 µg/disk), azithromycin (15 µg/disk), ciprofloxacin (5 µg/disk), erythromycin (15 µg/disk), gentamicin (10 µg/disk), streptomycin (10 µg/disk), tetracyclines (30 µg/disk), and

trimethoprime / sulfamethoxazole (25 µg/disk) were used. The agar disc diffusion method was adapted according to performance standards of CLSI (12), for assessing the antibacterial activity.

RESULTS AND DISCUSSION

The present study showed that Three different types of bacteria were isolated from a total of (105) faecal samples collected from diarrhoeic calves. Out of 105 faecal samples forty five (45) samples were found positive for *E. coli* giving positive reaction to lactose fermentation on MacConkey agar, metallic green sheen colonies on Eosin-Methylene Blue agar and yellowish green colonies on Brilliant Green Agar. Twenty two (22) samples were found positive for *Salmonella enterica* producing negative reaction to lactose fermentation on MacConkey agar. The organism produce opaque, translucent and colorless colonies on Salmonella- Shigella agar, pale pink colour colonies against a pinkish background on BGA and deep blue color from green colour simmon's citrate agar. Sixteen (16) samples were found positive for *staphylococcus aureus* producing yellowish colonies on Staphylococcus agar No. 110, hemolysis on Blood agar table (1,2,3). The results of frequency distribution of bacterial isolates are presented in table (4) Out of (105) faecal samples, 45 (42.85%) samples were belong to for *E. coli*, 22 (20.95%) samples were positive for *Salmonella spp*., 16 (15.23%) samples were positive for *Staphylococcus spp*., 12 (11.42%) samples were accounted for mixed infection and 10 (9.52%) samples were negative for bacteria. Isolated *E.coli* gate higher percentage than *Salmonella spp.* and *Staphylococcus spp.* The frequency distributions of different species of bacterial isolates in different faecal samples were found variable. In this study there was significant relation between types of pathogenic bacteria which were in agreement with findings of Aggernesh, 2010 (2) and Dereje, 2012 (14). When the prevalence of *E. coli* among different types of calf diarrhea is considered, it was recovered at highest rate from watery diarrhea. In the present findings diarrhea was highly associated with age of calves. Accordingly, young calves below 2 weeks were at high risk of being affected with diarrhea. This could be due to a number of factors. All the farms assessed administer colostrums to new born calves. However, the efficiency of colostrums intake and gut absorption may be affected by farm management practices.

Table 1. Characterization of isolated bacterial pathogens from diarrhoeic calves by Gram's staining technique

Identification	Gram's Staining		
	Shape	Gram's Staining Reaction(-/+)	Arrangement
E.coli	**Short plump rods**	**Gram negative**	**Single, paired or in short chain**
Salmonella spp.	**Very short plump rods**	**Gram negative**	**Single**
Staphylococcus spp	**Cocci arranged**	**Gram-positive**	**Grape-like clusters**

Table 2. Characterization of isolated bacterial pathogens by cultural properties

Name of Culture Media Used	Observation *E. Coli*	*Salmonella spp*	*Staphylococcus spp*
Nutrient Agar	Smooth, circular, white to grayish colony with peculiar fetid odour	Small, round and smooth colony	Growth of circular, small smooth, convex, and golden yellowish colonies
Blood Agar	Produce haemolysis	Produce haemolysis	Produce haemolysis
Brilliant Green	Yellowish green	Pinkish white	No growth (-)
Staphylococcus No.10	No growth (-)	No growth (-)	Yellowish color colony
Mac Conkey Agar	Rose pink lactose Fermenter colony.	Colourless, pale, translucent colony.	No growth (-)
Eosin-Methylene Blue (Emb) Agar	Moist circular colonies with dark centers yellow green metallic sheen	No growth (-)	No growth (-)
Salmonella- Shigella	Pink colour colony	Translucent colourless smooth colony	No growth (-)
Simmons Citrate	No growth (-)	Deep blue colour	No growth (-)
Xylose-Lysine-Desoxycholate	Yellow to yellow red colonies	Red colonies with black centers	No growth to slight growth
Mannitol Salt	No growth (-)	No growth (-)	yellow colonies

Table 3. Characterization by biochemical reactions of *E. coli, Salmonella spp*. and *Staphylococcus* spp

Isolated Organism	Oxidase Test	Catalase tes	Indole Test	Methyl-Red Test	Voges-Poskauer Reaction	Citrate Utilization Test	MIU Test	TSI Test	Hydrogen sulphide
E. coli	_	+	+	+	_	_	+ All	Butt-Y Slant-Y	_
Salmonella spp.	_	+	_	+	_	_	+	Butt-Y Slant-R	+
Staphylococcus spp.	_	+	_	+	_	_	_	Butt-Y Slant-Y	+

:no reaction; +:reactive ; Butt: buttom; Y: yellow; R: red

Table 4. Frequency of distribution of positive *E. coli, Salmonella* spp. and *Staphylococcous* spp.

Total Number of Samples Examined	Name of Isolated Bacteria	Total Number of Positive Samples	Frequency of Distribution (%)
105	*E. coli*	45	42.85%
	Salmonella spp.	22	20.95%
	Staphylococcus	16	15.23%
	Mixed infection involved	12	11.42%
	Negative for bacteria	10	9.52%
Chi- Square	----	----	10.037 **

** (P<0.01).

The result can be associated with many factors, at younger age; delay in first colostrums feeding was associated with higher risk of being affected with diarrhea. The finding that delayed colostrums intake (latter than 6 hours of age) associated with high risk of being affected with diarrhea agrees with other reports. Olsson *et al*. 1993 (30) found that each hour of delay in colostrums ingestion in the first 12 hours of age increased the chance of a calf becoming ill by 10%. Matte *et al*. 1982 (25) found that 61% of colostral

immunoglobulin containing 80mg/ml of IgG is absorbed in six hours and decreases sharply thereafter. This indicates that the first six hours are the period in which maximum absorption of colostral immunoglobulin takes place (16). Therefore, delays in administration could lead to lack of colostrum originated from originated maternal antibodies to protect calves from enteric pathogens.It is also indicated that the risk of failure of passive immunity transfer in bottle feeding is greater than in naturally suckled calves because of intake of inadequate colostrums volume and IgG and the mothering effect doesn't provide suitable gain to advocate leaving calves with the dam. But during bottle fed the colostrums might be contaminated with many environmental pathogens due to careless management systems. Results of the present study indicated that all the three different types of bacteria were not present in the same faecal sample collected from diarrhoeic calves. The incidence of different types of bacteria isolated from calf diarrhoea, correlate with the findings of (34). Haque and samad (19) isolated 9.61% *Salmonella* from calves, Joon and Kaura (22) isolated 23(23%) *E. coli* and 5 (5%) *Salmonella* from 100 fecal samples. Oporto *et al*. (31) stated that the prevalence of *E. coli* in bovine herds was 35.9%, Valdivia-Andy *et al.,* (37) found verotoxin producing *E. coli* in 63.7% of the samples tested, Bendali *et al*. (7) isolated 20.3% *E. coli* from fecal samples of diarrhoeic calves, and Khan and Khan (23) isolated enteropathogenic *E.coli* (54-58%), *Staphylococcus* (7-10%) and *Salmonella (*13-14%).

Antibacterial activity against isolated

From all isolates studied, the antimicrobial resistance pattern was summarized in Table (5, 6 and 7). A total of 45 isolates of *E. coli*, 22 *Salmonella spp*. and 16 *Staphylococcus spp* demonstrated resistance to at least two of the tested antimicrobials. 100% of *E. coli* strains, *Salmonella spp*. and *Staphylococcus spp* were resistant to ampicillin, and amoxicillin. Additionally, 82% - 88% of *E. coli* strains, 81% - 86% of *Salmonella spp*. and 81% -87% of *Staphylococcus spp* were resistant to tetracycline, streptomycin and gentamicin. However, nearly 26% - 33% of *E. coli* strains, 36% - 40% of *Salmonella spp*. and 31% -37% of *Staphylococcus spp* were moderate sensitivity to trimethoprim / Sulfamethoxazole, and erythromycin. Additionally, still some bacterial isolates 26% - 66% of *E. coli* strains, 45% - 72% of *Salmonella spp*. and 62% -75% of *Staphylococcus spp*. were sensitive to azithromycin and ciprofloxacin. These results suggested that the antimicrobial resistance of the *E. coli*, *Salmonella spp*. and *Staphylococcus spp***.** might be derived from the excessive use of antimicrobials of their hosts. This study showed that antimicrobial resistance is widespread in enteric *E. coli*, *Salmonella* spp. and *Staphylococcus spp*. from diarrheic preweaned dairy calves. Ampicillin, amoxycillin, erythromycin, streptomycin or tetracyclines were the most prevalent resistance traits and isolates resistant to all these antimicrobials were common. The findings are in agreement with studies in preweaned dairy calves from other countries (8,35). However, most of the *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. were susceptible to azithromycin, ciprofloxacin. These findings satisfy the result of (3, 17,28) who stated that calf isolates were highly sensitive to ciprofloxacin, levofloxacin and resistant to ampicillin, erythromycin, gentamicin and amoxicillin. Also quite similar results were obtained from (32,22) who reported that most of the bacteria isolated from calf diarrhoea were less sensitive to tetracycline, chloramphenical, streptomycin and moderately sensitive to ampicillin, amoxicillin, penicillin gentamycin and kanamycin. The variation in the sensitivity of antibiotics of the faecal isolates may be due to the outcome of choice and also the indiscriminate use of antibiotic in different disease stage to various species of animals. In such calves a high occurrence of resistance can be anticipated since a large proportion of the animals are probably treated with antimicrobials. An equally high prevalence of resistant *E. coli , Salmonella* spp. and *Staphylococcus spp* in the untreated calves of the present study is therefore remarkable. Results of the current study evidence obtained from laboratory and epidemiological studies indicated that the persistence of resistant bacteria was related to the persistence of

antimicrobial drug use (5). If an antimicrobial drug is used, continuously, the persistence of resistant organisms will go on. Thus, *E. coli* has often higher degrees of antimicrobials resistance which have a long history of use (4). Series of studies on the resistance of *E. coli* which were isolated from animals and humans strongly suggested that those bacteria which are resistant to antimicrobials used in animals would also be resistant to antimicrobials used in humans (27, 36).

Conclusion: E.coli recorded highly frequency from isolated bacteria, All isolated pathogenic bacteria were resistant against bacterial antibiotics

Table 6. Antibacterial activity against isolated *E. coli* (n= 45).

Antimicrobial agent	No (%) of *E. coli*			Chi- Square
	Susceptible	Intermediate	Resistant	
Ampicillin	0 (0)	0 (0)	45 (100)	15.00 **
Amoxicillin	0 (0)	0 (0)	45 (100)	15.00 **
Azithromycin	12 (26.6)	0 (0)	33 (73.33)	12.67 **
Ciprofloxacin	30 (66.66)	0 (0)	15 (66.66)	12.46 **
Erythromycin	0 (0)	15 (33.33)	12(73.33)	13.07 **
Gentamicin	7 (15.55)	0 (0)	40 (88.88)	14.26 **
Streptomycin	2 (4.4)	0 (0)	43(84.45)	13.52 **
Tetracycline	8 (17.77)	0 (0)	38 (82.23)	13.39 **
Trimethoprim / Sulfamethoxazole	0 (0)	12 (26.6)	33 (73.33)	12.67 **
Chi- Square	14.65 **	9.72 **	9.16 **	---

** (P<0.01).

Table 7. Antibacterial activity against isolated *Salmonella spp* (n=22).

Antimicrobial agent	No (%) of *Salmonella spp*			Chi- Square
	Susceptible	Intermediate	Resistant	
Ampicillin	0 (0)	0 (0)	22 (100)	15.00 **
Amoxicillin	0 (0)	0 (0)	22 (100)	15.00 **
Azithromycin	12 (54.54)	2 (9.09)	8 (36.36)	10.62 **
Ciprofloxacin	16 (72.72)	0 (0)	6 (27.27)	12.58 **
Erythromycin	0 (0)	9 (40.90)	13 (59.10)	12.03 **
Gentamicin	3 (13.63)	0 (0)	19 (86.37)	13.94 **
Streptomycin	3 (13.63)	0 (0)	19 (86.37)	13.68 **
Tetracycline	4 (18.18)	0 (0)	18 (81.82)	13.41 **
Trimethoprime / Sulfamethoxazole	0 (0)	8 (36.36)	14 (63.64)	11.73 **
Chi- Square	13.92 **	10.81 **	11.35 **	---

** (P<0.01).

Table 8. Antibacterial activity against isolated *Staphylococcus spp* (n = 16).

Antimicrobial agent	No (%) of *Staphylococcus spp*			Chi- Square
	Susceptible	Intermediate	Resistant	
Ampicillin	0 (0)	0 (0)	16 (100)	15.00 **
Amoxicillin	0 (0)	0 (0)	16 (100)	15.00 **
Azithromycin	10 (62.5)	0 (0)	6 (37.5)	12.57 **
Ciprofloxacin	12 (75)	0 (0)	4 (25)	13.54 **
Erythromycin	0 (0)	6 (37.5)	10 (62.5)	12.57 **
Gentamicin	2 (12.5)	0 (0)	14 (87.5)	13.92 **
Streptomycin	2 (12.5)	0 (0)	14 (87.5)	13.92 **
Tetracycline	3 (18.75)	0 (0)	13 (81.25)	13.26 **
Trimethoprim / Sulfamethoxazole	0 (12.5)	5 (31.25)	11 (68.75)	11.75 **
Chi- Square	13.53 **	9.15 **	11.82 **	---

** (P<0.01).

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