Research Article

Multidrug Resistance Pattern of *Enterobacter* Spp. Isolated from Acute Respiratory Tract Infected Camels (*Camelus dromedarius*)

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Abstract | The genus *Enterobacter* is a gram negative commensal coliform member of *Enterobacteriaceae*. In immunocompromised animals *Enterobacter* become opportunistic pathogen and severely affects therapeutic management of infections by evading proper effects of antibiotics. Thus the present study was designed to determine resistance pattern of *Enterobacter* obtained from acute respiratory tract infected camels (*Camelus dromedarius*). Total 16 *Enterobacter* spp. isolates were obtained from 46 deep nasal discharge samples of acute respiratory tract infected camels and preliminarily confirmed on the basis of IMViC pattern, hemolysis and sugar fermentation pattern. Confirmed isolates were screened for susceptibility against 25 antibiotics of various groups. All isolates were showed multidrug resistance pattern and 100% isolates were resistant to ampicillin, bacitracin, erythromycin, clindamycin, rifampicin, vancomycin and oxacillin. While all isolates were sensitive to gentamicin and imepenem and in decreasing order isolates were showing variable percentage of sensitivity for cefepime and ciprofloxacin (93.75%), norfloxacin and cefotaxime (87.50%), ceftazidime and co-trimoxazole (81.25%), colistin (68.75%), chloramphenicol (62.50%), kanamycin and trimethoprim (56.25%), tetracycline (31.25%) and cephalothin (25%) isolates were sensitive.

Keywords | Multidrug resistance, *Enterobacter*, Acute respiratory tract infection, Camel

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INTRODUCTION

Camel *(Camelus dromedarius)* is a state animal of Rajasthan and has significant role in economy of poor farmers through various ways such as camel milk, wool, riding and draft. Camel has also important role in agricultural utilities along with especial adaptations for Rajasthan desert but in certain harsh condition like sudden environment changes make it susceptible for opportunistic infections. Pulmonary diseases are among the emerging problems of camels that are causing considerable loss in production and death (Njage et al., 2012; Al-Juboori et al., 2013). Genus *Enterobacter* is more specifically a nosocomial opportunistic pathogen and is sought out to be one of the many key causes for extra intestinal infections next to *Escherichia coli* (Osterblad et al., 1999). In the 1970s, *Enterobacter* was first noted as a common cause of nosocomial infections in immuno-compromised hosts with respiratory, urinary, and gastrointestinal

tracts infections (Wilberger et al., 2012). Thus it was observed that *Enterobacter* commonly associated with respiratory, gastrointestinal and urinary tract infections in addition to wound, bloodstream, and central nervous system infections (Bordeanu et al., 2012).

The physical examination of *Enterobacter* respiratory tract infections may include high fever, tachycardia, hypoxemia and cyanosis. Infected animal with pulmonary consolidation may present with crackling sounds, dullness to percussion, tubular breath sounds and egophony. Pleural effusion may manifest as dullness to percussion and decreased breath sounds (Edward and Ewing, 1972; Gohary et al., 2012). Since during the last decade *Enterobacter* has emerged as an important hospital pathogen responsible for nosocomial respiratory tract infections with exhibiting high resistance to broad-spectrum antibiotics and the emergence of extended-spectrum cephalosporin-resistant strains has been also documented (Thiolas et al., 2005). In addition to diseases causing, genus *Enterobacter* is also capable to acquire antibiotic resistance in short time with various mechanism such as Beta-lactamase & Extended-Spectrum Beta-lactamase (ESBL) production, multidrug efflux system, low outer membrane permeability, mutations in chromosomal genes and additional acquired resistance genes via plasmids, transposons and phage makes this organism highly resistant (Harbottle et al., 2006). Though multidrug resistance pattern of *Enterobacter* among human patients explored well but in animals especially camels it has not studied very much. Thus the present study was carried out to characterize genus *Enterobacter* from respiratory tract infection in camels on the basis of their biochemical properties and to determine multidrug resistance pattern of organism for prevention of further morbidity and mortality of animals because of resistant opportunistic pathogens.

MATERIAL AND METHOD

Isolation and identification

A total of 46 samples of deep nasal discharge from acute respiratory tract infected camels were collected aseptically with sterile absorbent swabs soaked in nutrient broth. All samples has been collected from clinical complex of college of veterinary and animal science, Bikaner (Rajasthan) India on the basis of clinical symptoms of acute respiratory tract infection without discrimination of age, sex and breed of camels. The samples were inoculated on nutrient agar plates and then processed for isolation and identification of *Enterobacter* spp. (Cowan and Steel 1974; Quinn et al., 1994). Out of the 46 samples 16 suspected isolates were proceeded on the basis of phenotypic and biochemical properties such as cultural characteristics, motility, lactose fermentation, IMViC pattern, H_2S production in TSI agar, lysine decarboxylase and urease activity.

Antibiotic sensitivity test

To determine the antibiogram of the isolates against various 25 antibiotics (Table 1) the method of Bauer et al. (1966) was followed. The isolates were inoculated in sterile 5 ml nutrient broth tubes and incubated for 18 hour at 37°C. The opacity was adjusted to 0.5 McFarland opacity standards (Quinn et al., 1994) and inoculums were well spread over the agar surface with the help of sterilized swab. Plates were allowed to dry for 10 minute at 37°C and then antibiotic discs (Hi Media, Mumbai) were carefully placed on the surface with enough space around each disc for diffusion of the antibiotic. Plates were incubated for 24 hour at 37°C and the diameter of zone of inhibition of growth around each disc was measured in millimeters. After inhibition zone measurement, result interpretation was made with standard chart provided by disc manufacturer (Hi Media, Mumbai).

RESULTS

In the present investigation 16 *Enterobacter* spp. were isolated from 46 nasal samples from acute respiratory tract infected camels on the basis of their phenotypic and biochemical properties. All the isolates showed pink lactose fermenting, non- metallic sheen, mucoid colonies on respective culture media with typical IMViC pattern (- - + +) and not produced H_2S gas in Triple Sugar Iron (TSI) agar. Typically all isolates were motile, urease positive and negative for lysine decarboxylation. In antibiogram study, all isolates were 100% sensitive to gentamicin and imipenem while completely resistant to ampicillin, bacitracin, erythromycin, clindamycin, rifampicin, vancomycin and oxacillin. All *Enterobacters* showed multidrug resistance pattern with minimum resistance to fifteen antibiotics. The studied isolates were sensitive with following decreasing percentage such as cefepime & ciprofloxacin (93.75%), norfloxacin & cefotaxime (87.50%), ceftazidime & co-trimoxazole (81.25%) and 56.25%

Table 1: Antibiogram of *Enterobacter* spp. obtained from acute respiratory tract infected camels

S. No.	Antibiogram disc	Conc. (mcg/disc)	Percent (Number of isolates)		
			Sensitive	Intermediate	Resistant
1	Gentamicin (G)	120	100 (16)	-	-
2	Imipenem (I)	10	100 (16)	-	-
3	Cefepime (Cpm)	30	93.75 (15)	-	6.25 (1)
4	Ciprofloxacin (Cf)	5	93.75 (15)	6.25 (1)	-
5	Norfloxacin (Nx)	10	87.50 (14)	6.25 (1)	6.25 (1)
6	Cefotaxime (Ce)	30	87.50 (14)	-	12.50 (2)
7	Ceftazidime (Ca)	30	81.25 (13)	12.50 (2)	6.25 (1)
8	Co-trimoxazole (Co)	23.75/1.25	81.25 (13)	6.25 (1)	12.50 (2)
9	Colistin (Cl)	10	68.75 (11)	6.25 (1)	25 (4)
10	Chloramphenicol (C)	30	62.50 (10)	-	37.50 (6)
11	Kanamycin (K)	30	56.25 (9)	-	43.75 (7)
12	Trimethoprim (Tr)	5	56.25 (9)	18.75 (3)	25 (4)
13	Tetracycline (T)	30	31.25 (5)	12.50 (2)	56.25 (9)
14	Cephalothin (Ch)	30	25 (4)	12.50 (2)	62.50 (10)
15	Ampicillin/Sulbactum (A/s)	10/10	18.75 (3)	12.50 (2)	68.75 (11)
16	Nalidixic Acid (Na)	30	12.50 (2)	-	87.50 (14)
17	Cloxacillin (Cx)	10	6.25 (1)	56.25 (9)	37.50 (6)
18	Penicillin (P)	10 unit	6.25 (1)	-	93.75 (15)
19	Ampicillin (A)	10	-	-	100 (16)
20	Bacitracin (B)	10 Units	-	-	100 (16)
21	Erythromycin (E)	15	-	-	100 (16)
22	Clindamycin (Cd)	2	-	-	100 (16)
23	Rifampicin (R)	5	-	-	100 (16)
24	Vancomycin(Va)	30	-	-	100 (16)
25	Oxacillin(Ox)	1	-	-	100 (16)

isolates were resistant to tetracycline, 62.50% to cephalothin, 68.75% to ampicillin/sulbactum, 87.50% to nalidixic acid and 93.75% isolates were resistant to penicillin. All other antibiotics showed variable efficacy as described in table 1.

DISCUSSION

In the present study, *Enterobacter* spp. showed typical biochemical and cultural phenotypic characteristic as described in literature (Edward and Ewing, 1972; Cowan and Steel, 1975). Since chromosomal DNA not so rapidly change in comparison to plasmid and most of phenotypic and cultural properties govern by chromosomal DNA so there may possibilities that *Enterobacter* existing long without any phenotypic variations (Holmes and Jobling, 1996). On the basis of several easily performed biochemical tests, Zabransky et al. (1969) and Iversen et al. (2006) has also characterize *Enterobacter spp.* obtained from clinical cases. Similar to present study they found biochemical characterization is a reproducible technique for epidemiological surveillance up to genus identification but for precise species differentiation further genotypes (DNA cluster groups based on partial 16SrDNA sequence analysis) characterization is required.

For antibiotic susceptibility pattern, present study also found similar observation without resistance to cefotaxime, aztreonam, imipenem, gentamicin, nalidixic

acid and ciprofloxacin (Osterblad et al., 1999) and in the study of Magnet et al. (2013), *Enterobacter* spp. were resistant to most of antibiotics, but were moderately sensitive (50%) to ciprofloxacin, tetracycline and doxycycline.

Observations in present study had accordance with the Al-Juboori et al. (2013), who revealed that the *Enterobacter* isolates from clinical and subclinical mastitis from camel milk showed moderate sensitivity to carbenicillin, streptomycin, sulphamethoxazole and gentamicin while less sensitive or even resistive towards ampicillin, colistin, penicillin G and tetracycline. Greenup and Blazevic, (1971) found slight variations that all the 28 strains of *Enterobacter* were sensitive towards gentamicin, chloramphenicol and nalidixic acid followed by sulfisoxazole, kanamycin, tetracycline, streptomycin and all were resistant to ampicillin. The *Enterobacter* was resistant to ampicillin (81.3%), chloramphenicol (75.0%), ciprofloxacin (6.3%), enrofloxacin (18.8%), neomycin (37.5%), norfloxacin (25.0%), streptomycin (56.3%) and tetracycline (75.0%) in the study of antimicrobial susceptibility of *Enterobacter aerogenes* from free-range chickens (Ojo et al., 2012) and the study by Nyenje et al. (2012) found that *Enterobacter cloacae* isolates registered 100% susceptibility to ciprofloxacin and various percentages of susceptibility was reported to chloramphenicol and gentamicin (91%) each, nalidixic acid (97%) and streptomycin (94%). In support of present study, these all variable patterns of resistance may prove that *Enterobacter* not only having variable mechanisms of antibiotic resistance but also has variable multidrug resistance patterns with different source of samples. It may understand that acquired antibiotic resistance may result from the mutation of normal cellular genes, the acquisition of foreign resistance genes, or a combination of these two mechanisms and these mechanisms most commonly governed by mobile genetic elements such as plasmids, transposons and integrons (Harbottle et al., 2006). The mobile genetic elements are most variable genetic material with organisms, environments and cross transmission conditions thus not only earlier studies but also present observed variable pattern of multidrug resistance among *Enterobacter* and these genetic component may also explain variation of antibiotic resistance with different source of samples, geographic regions and host animals (Ojo et al., 2012; Al-Juboori et al., 2013).

Although, less information is available regarding *Enterobacter* antibiotic resistance patterns in veterinary medicine; however, emergence of resistance to beta-lactam agents indicates indiscriminate and excessive use of these antibiotics in food producing animals and for therapeutic management to prevent various infections among animal population (Reisbig and Hanson 2004). According to veterinarians practicing in study area, tetracycline, norfloxacin and cephalosporin are more commonly prescribed antibiotics in comparison to gentamicin and yet imipenem is not in veterinary practice thus the usages of these antibiotics positively correlate with increased resistance among *Enterobacter* strains in this investigation. Wilberger et al. (2012) also found similar positive correlation of increased use of antibiotic and their resistance for enrofloxacin and gentamicin in the USA during study of antibiotic resistance among *Enterobacter* spp. isolated from infection in animals.

McEwen and Fedorka-Cray (2002) has also concluded that excessive use of antibiotics induces the selection of resistant strains by producing hydrolytic enzymes and decreasing the active drug concentration via the alteration of permeability in outer membranes and enhances persistence and dissemination of antibiotic resistance not only in hospitals but also in food chains and ecosystems. In the presence of above facts, present study may conclude that antibiotic resistance not only govern by various inherited and acquired mechanisms but also by indiscriminate use of antibiotic thus the present study suggest prudent and wise use of antibiotics and further molecular studies to find exact mechanism of antibiotic resistance along with genetic characterization of *Enterobacter* strains to curb mortality and morbidity due to resistant infections.

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