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ANTIBIOGRAM OF *ARCOBACTER* SPECIES ISOLATED FROM ANIMALS, FOODS OF ANIMAL ORIGIN AND HUMANS IN ANDHRA PRADESH, INDIA

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Abstract: *Arcobacter* is an emerging foodborne pathogen that have been associated with diseases of both humans and animals. A total of 41 *Arcobacter* isolates (16 *A. butzleri*, 13 *A. cryaerophilus* and 12 *A. skirrowii*) isolated from diverse sources like faecal swabs of livestock (21), raw foods of animal origin (13) and human stool samples (7) were subjected to antimicrobial susceptibility testing against ten different antibiotics by disc diffusion method. Antibiogram of *Arcobacter* isolates revealed sensitivity to tetracycline (100%), ciprofloxacin (95.1%) and gentamicin (82.9%). Higher resistance was observed for vancomycin (100%), co-trimoxazole (87.8%), chloramphenicol (78%) and erythromycin (51.2%) with remarkable intermediate resistance against kanamycin (68.2%), nalidixic acid (53.6%) and cefoxitin (43.9%). The present study highlighted alarming antimicrobial resistance in *Arcobacter* species of animal and human origin, which is of grave concern to animal and human health.

Keywords: *Arcobacter,* antibiogram, emerging foodborne pathogen, resistance.

Introduction

Foodborne zoonotic pathogens are of great importance with regard to consumer health and protection. Since the introduction of the genus *Arcobacter* in 1991, the association of *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* with humans and animals has been clearly established (Collado and Figueras, 2011). *Arcobacter* species have been associated with diseases of both humans (Samie *et al.,* 2007 and Jiang *et al.,* 2010) and animals (De Oliveira *et al.,* 1997) and are commonly isolated from food products of animal origin (Kabeya *et al.,* 2004 and Amare *et al.,* 2011), which has led to classification of *Arcobacter* species as emerging food pathogens. The International Commission on Microbiological Specifications for Foods categorized *A. butzleri* as a 'serious hazard' to human health (ICMSF, 2002).

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Studies on antimicrobial resistance of *Arcobacter* species are lacking although some preliminary studies have been done on antimicrobial sensitivity of *Arcobacter* species against certain antibiotics (Otth *et al.,* 2004 and Abdelbaqi *et al.,* 2007). Furthermore, many *Arcobacter* species isolated from humans, livestock and meat carcasses were found to be resistant to commonly used antimicrobials in human and veterinary medicine (Fera *et al.,* 2003, Kabeya *et al.,* 2004 and Zacharow *et al.,* 2015). Despite the increasing concern over this issue, reports regarding the antimicrobial resistance of *Arcobacter* species from India are very scarce (Mohan *et al.,* 2014).

Emerging era of "antimicrobial resistance" and "one world one health" issues have highlighted the importance of checking antimicrobial resistance in foodborne pathogens, so as to safeguard the health of humans and animals. Hence the present study was carried out with an objective of studying the antibiogram of *Arcobacter* species of animal and human origin in Andhra Pradesh, India.

Materials and methods

Reference strains: The reference strain of *A. butzleri* (ATCC 49616) used in the present study were obtained from Division of Veterinary Public Health, Indian Veterinary Research Institute, Izatnagar, India.

Bacterial isolates: A total of 41 *Arcobacter* isolates isolated from diverse sources like faecal swabs of livestock (21), raw foods of animal origin (13) and human stool samples (7) were used in this study. The identification of each isolate was carried out by using the following tests: Gram staining (Gram negative, short 'S' shaped rods), dark field microscopy (cork-screw motility), oxidase (positive), catalase (positive), nitrate reduction (positive) and hippurate hydrolysis (negative) (Vandamme *et al.,* 2005). Further, all the 41 isolates were confirmed at genus level as *Arcobacter* by genus specific PCR targeting 16S rRNA gene (Harmon and Wesley, 1996) and at species level as *A. butzleri* (16), *A. cryaerophilus* (13) and *A. skirrowii* (12) by multiplex PCR targeting 16S and 23S rDNA (Houf *et al.,* 2000). *Arcobacter* isolates from faecal swabs of livestock include those from pigs (8), chicken (6), turkey (2), cattle (2), sheep (2) and duck (1). *Arcobacter* isolates from raw foods of animal origin include those from chicken (5), pork (4), milk (2) and mutton (2). *Arcobacter* isolates from human stool samples include those from pig/poultry farm workers (3), veterinary students (2) and diarrhoeic humans (2).Whole cell DNA was extracted by boiling and snap chilling method (Ramees *et al.,* 2014).

Antimicrobial susceptibility testing: Antibiogram of *Arcobacter* species was carried out against 10 different antibiotics like Cefoxitin (CX, 30 µg), Chloramphenicol (C, 30 µg), Ciprofloxacin (CIP, 5 µg), Co-Trimoxazole (COT, 25 µg), Erythromycin (E, 15 µg), Gentamicin (GEN, 10 µg), Kanamycin (K, 30 µg), Nalidixic acid (NA, 30 µg), Tetracycline (TE, 30 µg) and Vancomycin (VA, 30 µg) by Kirby Bauer disc diffusion method (Bauer *et al.,* 1966). *Arcobacter* isolates were sub-cultured on *Arcobacter* blood agar plates and incubated for 48 h under micro-aerophilic conditions at 30°C. Direct colony suspension of each isolate was made in PBS (pH 7.4) and the turbidity was adjusted to 0.5 McFarland (equivalent to an approximate cell density of 1.5 x 10^8 CFU/ml)). About 200 µl of each inoculum was seeded on the Mueller Hinton (MH) agar supplemented with 5% defibrinized sheep blood using sterile cotton-tipped swab. Plates were allowed to dry and antibiotic discs were placed aseptically with sterile fine forceps. The plates were incubated at 30°C for 48 h under micro-aerophilic conditions. The diameter of inhibition zones was measured and susceptibility patterns of *Arcobacter* species were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2014).

Results and discussion

The emergence of multidrug resistance among food borne pathogens is a cause of grave concern to public health (Tiwari *et al.,* 2013). In this context, *in vitro* antibiotic sensitivity test was performed for a total of 41 *Arcobacter* strains isolated from diverse sources in Andhra Pradesh. It was found that all the *Arcobacter* isolates were resistant to at least one of the ten antibiotics tested. Most of the *Arcobacter* isolates showed sensitivity to tetracycline (100%), ciprofloxacin (95.1%) and gentamicin (82.9%). Higher resistance was observed for vancomycin (100%), co-trimoxazole (87.8%), chloramphenicol (78.0%) and erythromycin (51.2%). Notable percentage of isolates were intermediately resistant against kanamycin (68.2%), nalidixic acid (53.6%) and cefoxitin (43.9%) (Table 1). The pattern of drug resistance varied according to species of *Arcobacter* and origin of *Arcobacter* isolates. The species-wise and source-wise details of antibiotic resistance patterns were presented in Table 2 and 3, respectively.

A. butzleri isolates (n=16) were found completely resistant to vancomycin (16/16, 100%) followed by co-trimoxazole (15/16, 93.7%), chloramphenicol (13/16, 81.2%), erythromycin (8/16, 50%), cefoxitin (6/16, 37.5%), nalidixic acid (6/16, 37.5%), kanamycin (5/16, 31.2%) and gentamicin (3/16, 18.7%). All the *A. butzleri* isolates were found susceptible to ciprofloxacin and tetracycline. Likewise, *A. cryaerophilus* isolates were found completely

resistant to vancomycin (13/13, 100%) followed by co-trimoxazole (11/13, 84.6%), chloramphenicol (10/13, 76.9%), erythromycin (7/13, 53.8%), cefoxitin (5/13, 38.4%), nalidixic acid (4/13, 30.7%), kanamycin (3/13, 23%), gentamicin (1/13, 7.6%) and ciprofloxacin (1/13, 7.6%). All the *A. cryaerophilus* were found susceptible to tetracycline. Further, *A. skirrowii* isolates were found completely resistant to vancomycin (12/12, 100%) followed by co-trimoxazole (9/12, 75%), chloramphenicol (9/12, 75%), erythromycin (6/12, 50%), cefoxitin (4/12, 33.3%), nalidixic acid (3/12, 25%) and kanamycin (1/12, 8.3%). All the *A. skirrowii* isolates were found susceptible to gentamicin, ciprofloxacin and tetracycline (Table 2).

The presence of resistance to erythromycin (51.2%) and ciprofloxacin (2.4%) among *Arcobacter* isolates is a matter of concern, for the reason that these antimicrobials are commonly suggested as first-line of drugs for the treatment of *Campylobacteraceae* infections in humans (Houf *et al.,* 2004). In the present study, we found that 51.2% of the *Arcobacter* isolates were resistant to erythromycin, the preferred antibiotic for *Arcobacter* infection. The present results were in agreement with Son *et al.* (2007) who reported higher resistance to erythromycin (79%) in *Arcobacter* isolates. Increased erythromycin resistance in human and animal *Arcobacter* isolates was also reported by Houf *et al.* (2004). However, Vandenberg *et al.* (2006) found a lower resistance rate to erythromycin (21.6%) than the present study. Kabeya *et al.* (2003) in their study from Japan, reported that 53.5% (65 out of 122) of the *Arcobacter* isolates were resistant to nalidixic acid, quite in line with our finding of 53.6%. Also in a study by Son *et al.* (2007), *Arcobacter* isolates displayed higher resistance (77%) to nalidixic acid. High levels of resistance to vancomycin, co-trimoxazole and chloramphenicol observed in *Arcobacter* isolates of present study was in agreement with the findings of Fera *et al.* (2003) and Kabeya *et al.* (2003). On the other hand, like Vandenberg *et al.* (2006) and Mohan *et al.* (2014) we found that most of the *Arcobacter* isolates were susceptible to tetracycline, gentamicin and ciprofloxacin. Tetracycline and ciprofloxacin susceptibility was also determined by Kabeya *et al.* (2004) and Son *et al.* (2007) for *Arcobacter* isolates. In the present study, all the *A. butzleri* and *A. skirrowii* isolates were found to be susceptible to ciprofloxacin and tetracycline, while an *A. cryaerophilus* isolate recovered from poultry faeces was resistant to ciprofloxacin and two showed intermediate resistance. These results are comparable to those of Atabay and Aydin (2001), who reported 100% susceptibility of *A. butzleri* strains to fluoroquinolones and tetracyclines.

Table 1: Antibiotic sensitivity/resistance patterns of *Arcobacter* isolates

Antimicrobial agent (dose)	Pattern of antibiogram					
	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Tetracycline (30 µg)	41/41	100%	-	-	-	-
Ciprofloxacin (5 µg)	39/41	95.1%	2/41	4.87%	1/41	2.4%
Gentamicin (10 µg)	34/41	82.9%	3/41	7.31%	4/41	9.7%
Kanamycin (30 µg)	4/41	9.75%	28/41	68.2%	9/41	21.9%
Nalidixic acid (30 µg)	6/41	14.6%	22/41	53.6%	13/41	31.7%
Cefoxitin (30 µg)	8/41	19.5%	18/41	43.9%	15/41	36.5%
Erythromycin (15 µg)	13/41	31.7%	7/41	17.0%	21/41	51.2%
Chloramphenicol (30 µg)	2/41	4.87%	7/41	17.0%	32/41	78.0%
Co-trimoxazole (25 µg)	2/41	4.87%	3/41	7.3%	36/41	87.8%
Vancomycin (30 µg)	-	-	-	-	41/41	100%

Of the isolates from various sources, the highest frequency of antimicrobial resistance phenotypes were observed for *Arcobacter*s isolated from foods of animal origin, where all the isolates were found resistant to vancomycin (100%) followed by chloramphenicol (92.3%), co-trimoxazole (84.6%), erythromycin (61.5%), cefoxitin (46.1%), kanamycin (38.4%), gentamicin and nalidixic acid (each 30.7%). All the isolates from livestock faecal origin were resistant to vancomycin (100%) followed by co-trimoxazole (80.9%), chloramphenicol (71.4%), nalidixic acid (42.8%), erythromycin (38.0%), cefoxitin (19.0%) and ciprofloxacin (4.7%). All the human isolates were resistant to co-trimoxazole and vancomycin (100%) followed by chloramphenicol, erythromycin and cefoxitin (71.4%) and kanamycin (57.1%) (Table 3).

A recent study that investigated the Minimal Inhibitory Concentration (MIC) of 43 *A. butzleri* strains recovered from various sources in Portugal (Ferreira *et al*., 2013) showed resistance in 55.8% of strains for ciprofloxacin as well as in 97.7% to 100% of strains for vancomycin and co-trimoxazole. At the same time, the only effective antibiotic reported by them was gentamicin. Shah *et al.* (2012) evaluated the resistance to antibiotics of several strains recovered from cattle, beef, milk and water using a disk diffusion method and determining the MIC by serial dilution, where only 6.5% of the tested strains showed resistance to tetracycline, 21.7% to ciprofloxacin and 26.1% to gentamicin. However, more strains showed resistance to erythromycin (69.6%). Resistance to quinolones has been linked to the use of this kind of antibiotic in livestock for preventing infections (Kayman *et al.,* 2012). On that point, a mutation in the quinolones resistance-determining region of the *gyrA* gene has been

shown to produce high levels of resistance in *Arcobacter* species (Collado and Figueras, 2011).

Table 2: Antibiotic resistance among *Arcobacter* isolates (species-wise)

Species	Resistant strains / No. of strains examined									
	TE	**CIP**	**GEN**	**K**	**NA**	**CX**	**E**	**C**	**COT**	**VA**
1. A. butzleri										
PF (2)	0/2	0/2	0/2	0/2	1/2	0/2	0/2	2/2	2/2	2/2
SF (2)	0/2	0/2	0/2	0/2	2/2	0/2	1/2	1/2	2/2	2/2
CF (1)	0/1	0/1	0/1	0/1	1/1	0/1	0/1	1/1	0/1	1/1
CM (2)	0/2	0/2	1/2	1/2	0/2	0/2	1/2	2/2	2/2	2/2
PK (1)	0/1	0/1	1/1	0/1	1/1	0/1	1/1	1/1	1/1	1/1
MK (1)	0/1	0/1	1/1	0/1	1/1	1/1	0/1	1/1	1/1	1/1
VS (2)	0/2	0/2	0/2	0/2	0/2	1/2	1/2	1/2	2/2	2/2
FW (3)	0/3	0/3	0/3	2/3	0/3	2/3	2/3	2/3	3/3	3/3
DH (2)	0/2	0/2	0/2	2/2	0/2	2/2	2/2	2/2	2/2	2/2
TOTAL (16)	**0/16 (0%)**	**0/16 (0%)**	**3/16 (18.7%)**	**5/16 (31.2%)**	**6/16 (37.5%)**	**6/16 (37.5%)**	**8/16 (50%)**	**13/16 (81.2%)**	**15/16 (93.7%)**	**16/16 (100%)**
2. A. cryaerophilus										
PF (2)	0/2	1/2	0/2	0/2	1/2	0/2	1/2	1/2	2/2	2/2
SF (3)	0/3	0/3	0/3	0/3	2/3	1/3	1/3	2/3	3/3	3/3
CF (1)	0/1	0/1	0/1	0/1	0/1	0/1	1/1	1/1	1/1	1/1
CM (3)	0/3	0/3	0/3	1/3	0/3	1/3	2/3	2/3	2/3	3/3
PK (3)	0/3	0/3	0/3	1/3	0/3	2/3	1/3	3/3	2/3	3/3
MK (1)	0/1	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
TOTAL (13)	**0/13 (0%)**	**1/13 (7.6 %)**	**1/13 (7.6%)**	**3/13 (23.0%)**	**4/13 (30.7%)**	**5/13 (38.4%)**	**7/13 (53.8%)**	**10/13 (76.9%)**	**11/13 (84.6%)**	**13/13 (100%)**
3. A. skirrowii										
PF (5)	0/5	0/5	0/5	0/5	1/5	2/5	2/5	3/5	2/5	5/5
SF (3)	0/3	0/3	0/3	0/3	1/3	0/3	1/3	2/3	3/3	3/3
SH (2)	0/2	0/2	0/2	0/2	0/2	1/2	1/2	2/2	2/2	2/2
MN (2)	0/2	0/2	0/2	1/2	1/2	1/2	2/2	2/2	2/2	2/2
TOTAL (12)	**0/12 (0%)**	**0/12 (0%)**	**0/12 (0%)**	**1/12 (8.3%)**	**3/12 (25%)**	**4/12 (33.3%)**	**6/12 (50%)**	**9/12 (75%)**	**9/12 (75%)**	**12/12 (100%)**
GRAND TOTAL	**0/41 (0%)**	**1/41 (2.4%)**	**4/41 (9.7%)**	**9/41 (21.9%)**	**13/41 (31.7%)**	**15/41 (36.5%)**	**21/41 (51.2%)**	**32/41 (78.0%)**	**35/41 (85.3%)**	**41/41 (100%)**

PF-poultry faeces, **SF**-pig faeces, **CF**-cattle faeces, **SH**-sheep faeces, **CM**-chicken meat, **PK**-pork, **MK**-milk, **MN**-mutton, **VS**-veterinary students, **FW**-farm workers, **DH**-diarrhoeic humans; **TE**-tetracycline, **CIP-**ciprofloxacin, **GEN**-gentamicin, **K**-kanamycin, **NA**-nalidixic acid, **CX**-cefoxitin, **E**-erythromycin, **C**-chloramphenicol, **COT**-co-trimoxazole, **VA**-vancomycin

Table 3: Antibiotic resistance among *Arcobacter* isolates (source-wise)

Source	Resistant strains / No. of strains examined									
	TE	**CIP**	**GEN**	**K**	**NA**	**CX**	**E**	**C**	**COT**	**VA**
1. FAECAL SWABS										
PF (9)	0/9	1/9	0/9	0/9	3/9	2/9	3/9	6/9	6/9	9/9
SF (8)	0/8	0/8	0/8	0/8	5/8	1/8	3/8	5/8	8/8	8/8
CF (2)	0/2	0/2	0/2	0/2	1/2	0/2	1/2	2/2	1/2	2/2
SH (2)	0/2	0/2	0/2	0/2	0/2	1/2	1/2	2/2	2/2	2/2
TOTAL (21)	**0/21 (0%)**	**1/21 (4.7 %)**	**0/21 (0%)**	**0/21 (0%)**	**9/21 (42.8 %)**	**4/21 (19.0%)**	**8/21 (38.0 %)**	**15/21 (71.4 %)**	**17/21 (80.9 %)**	**21/21 (100%)**
2. FOODS OF ANIMAL ORIGIN										
CM (5)	0/5	0/5	1/5	2/5	0/5	1/5	3/5	4/5	4/5	5/5
PK (4)	0/4	0/4	1/4	1/4	1/4	2/4	2/4	4/4	3/4	4/4
MK (2)	0/2	0/2	2/2	1/2	2/2	2/2	1/2	2/2	2/2	2/2
MN (2)	0/2	0/2	0/2	1/2	1/2	1/2	2/2	2/2	2/2	2/2
TOTAL (13)	**0/13 (0%)**	**0/13 (0%)**	**4/13 (30.7 %)**	**5/13 (38.4 %)**	**4/13 (30.7 %)**	**6/13 (46.1%)**	**8/13 (61.5 %)**	**12/13 (92.3 %)**	**11/13 (84.6 %)**	**13/13 (100%)**
3. HUMAN STOOL SAMPLES										
VS (2)	0/2	0/2	0/2	0/2	0/2	1/2	1/2	1/2	2/2	2/2
FW (3)	0/3	0/3	0/3	2/3	0/3	2/3	2/3	2/3	3/3	3/3
DH (2)	0/2	0/2	0/2	2/2	0/2	2/2	2/2	2/2	2/2	2/2
TOTAL (7)	**0/7 (0%)**	**0/7 (0%)**	**0/7 (0%)**	**4/7 (57.1 %)**	**0/7 (0%)**	**5/7 (71.4%)**	**5/7 (71.4 %)**	**5/7 (71.4 %)**	**7/7 (100%)**	**7/7 (100%)**
GRAND TOTAL	**0/41 (0%)**	**1/41 (2.4 %)**	**4/41 (9.7%)**	**9/41 (21.9 %)**	**13/41 (31.7 %)**	**15/41 (36.5%)**	**21/41 (51.2 %)**	**32/41 (78.0 %)**	**35/41 (85.3 %)**	**41/41 (100%)**

PF-poultry faeces, **SF**-pig faeces, **CF**-cattle faeces, **SH**-sheep faeces, **CM**-chicken meat, **PK**-pork, **MK**-milk, **MN**-mutton, **VS**-veterinary students, **FW**-farm workers, **DH**-diarrhoeic humans; **TE**-tetracycline, **CIP-**ciprofloxacin, **GEN**-gentamicin, **K**-kanamycin, **NA**-nalidixic acid, **CX**-cefoxitin, **E**-erythromycin, **C**-chloramphenicol, **COT**-co-trimoxazole, **VA**-vancomycin.

The differences among resistance patterns of *Arcobacter* isolates of animal and human origin observed in the present study could be related to the different antibiotic regimes used for different antimicrobial agents in livestock species and humans. Wide antimicrobial resistance pattern observed in the present investigation was possibly a consequence of extensive usage of these antibiotics in the treatment of livestock or in other sources e.g. contact with animal faeces, water sources which may be a cause of the transmission of resistance genes from various vectors to food producing animals.

Conclusion

The results from this study showed alarming resistance frequencies in *Arcobacter* isolates from animals, foods of animal origin and humans. Antibiotic resistance patterns in this study also revealed clear variations among resistance patterns between human and animal isolates. Some strains which showed resistance to more than eight antimicrobial agents tested is an alert for consumers who eat improperly cooked meat.

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