RESEARCH ARTICLE

Anti-Microbial Susceptibility Pattern of Extended Spectrum Beta-Lactamase (ESBL) Producing *Klebsiella* spp. Isolated from Chevon Marketed in Anand, Gujarat

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Abstract

The present study was intended to isolate extended-spectrum beta-lactamase (ESBL) producing *Klebsiella* spp. from chevon samples (n = 150) collected various retail chevon markets in and around Anand (Gujarat) to check their prevalence and evaluate the hygienic level. The isolates obtained were also subjected to anti-microbial susceptibility testing. According to Clinical and Laboratory Standards Institute (CLSI) guidelines, Bacterial isolation, identification, and anti-microbial susceptibility tests were performed. Out of 150 samples, *Klebsiella* spp. were recovered from 85 chevon samples with an overall prevalence of 56.67%. The highest prevalence was observed in the intestine (76.67%), while the lowest was observed in muscle samples (36.67%) with intermediate prevalence in other samples like heart, liver, and water. Amongst *Klebsiella* spp. isolates, 100 % antibiotic resistance was detected towards ampicillin and ceftazidime, followed by cefepime (64%) and ceftazidime/clavulinic acid (63%); whereas higher sensitivity was detected towards amoxicillin/clavulanic acid (89.41%) followed by gentamicin (70.58%) and amikacin (61.17%). The high prevalence of ELBS producing *Klebsiella* spp. with greater anti-microbial resistance suggest the judicious use of antibiotics and the need to track and monitor the spread of ESBL producing strains in food-producing animals to safeguard public health.

Keywords: Antibiotics resistance, Chevon, ESBL, *Klebsiella* spp. Prevalence.

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Introduction

In several countries, including India, consumers mostly prefer chevon, and it is widely eaten throughout the world. Chevon has lower saturated fat and cholesterol content (Madruga and Bressan, 2011) but a higher polyunsaturated fatty acid (PUFA) as compared to other red meats such as beef and lamb (Anaeto *et al*., 2010; Aghwan *et al*., 2014). As well-being, cognizant customers are nowadays inclined towards less fatty and more advantageous chevon. Further, the prominence of chevon on the worldwide meat advertise is expanding because of a direct consequence of its leanness and well-being - advantageous unsaturated fatty acid profile (Ivanovic *et al*., 2016; Anaeto *et al*., 2010).

Klebsiella species are facultatively anaerobic, gram-negative, non-motile, usually encapsulated rod-shaped bacteria. The genus Klebsiella is a member of *Enterobacteriaceae* family. Moreover, *Klebsiella* species known as the reservoir for antibiotic-resistant genes can spread to other Gram-negative bacteria. Due to the rise in the number of infections and increasing strains resistant to antibiotics, *K. pneumoniae* is gaining attention. According to the results reported to European Centre for Disease Prevention and Control (2018), more than 33% of the *K. pneumoniae* isolates were resistant to at least one anti-microbial group, the combined resistance to fluoroquinolones, third-generation cephalosporins, and aminoglycosides being the most common resistance phenotype. Because of the emergence of a multidrug, hypervirulent *K. pneumoniae* clone causes untreatable infections in healthy individuals (Lam *et al*., 2018; MC Lam *et al*., 2018).

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The ESBLs are plasmid-mediated bacterial enzymes of heterogeneous groups (Canton and Coque, 2006). ESBL enzymes can hydrolyze various β-lactam antibiotics like penicillin third and fourth generation cephalosporin and thus mediate resistance against these antibiotics (Saravanan *et al*., 2018). Nowadays, there is also an increasing therapeutic failure due to emergence of anti-microbial resistance and complications leading to very serious health issues as ESBL enzymes particularly produced by *Enterobacteriaceae* family, *E. coli* and *Klebsiella* spp., have developed effective defense mechanisms (Munita and Arias, 2016) against most of the antibiotics by causing lysis of the beta-lactam ring. Therefore, the present study was designed to investigate the prevalence and anti-microbial susceptibility pattern of ESBL producing *Klebsiella* spp. from chevon of retail markets in Anand, Gujarat.

Materials And Methods

Sample Collection

A total of 150 samples comprising muscles (n = 30), intestine (n = 30), liver (n = 30), heart (n = 30), and water samples (n = 30), were collected from various retail chevon markets in and around Anand (Gujarat). The samples were collected under aseptic precautions in sterile screw lid sample collectors and transported to the laboratory in an icebox for further processing and bacteriological investigation.

Enrichment of Samples and Selective Plating on Culture Media

Isolation of *Klebsiella* spp. was attempted from different samples of chevon according to the method described by Singh (1997). Each sample was first inoculated in 5 mL of nutrient broth for bacterial growth for 24 h at 37°C. The growth in nutrient broth was transferred to Mac-Conkey agar and again incubated at 37°C for 24 h. The pink-colored mucoid colonies (Fig. 1) were picked up and subcultured on HiCrome *Klebsiella* selective agar supplement (Carbenicillin 25 mg/500 mL) at 37°C for 24 to 48 h for specific isolation of *Klebsiella* spp. Purple-magenta-coloured mucoid colonies (Fig. 2) were considered to be typical *Klebsiella* spp. These colonies were further identified by their morphological characteristics, staining characteristics, and biochemical properties.

Anti-Microbial Susceptibility Test

Anti-microbial susceptibility tests were done on Muller-Hinton Agar (Hi-media lab) using the Kirby-Bauer disk diffusion method (Bauer *et al.,* 1996). The anti-microbial agents such as imipenem (IPM-10µg), gentamicin (GEN-10 µg), ampicillin (AMP-10 µg), amikacin (AK -30 µg), ceftazidime (CAZ-30 µg), ceftazidime/clavulinic acid (CAC-30/10 µg), ceftriaxone (CTR-30 µg), ciprofloxacin (CIP-30 µg), cefepime (CIP-50 µg) and amoxycillin/clavulanic acid (AMC-20/10 µg) were used for this test. The results were interpreted according to CLSI guidelines (CLSI, 2007).

Results and Discussion

Prevalence of *Klebsiella* spp.

Out of 150 samples cultured, 85 (56.67%) samples were found to be positive for *Klebsiella* spp. in chevon. Among the different sources of samples, the highest prevalence was observed in the intestine (76.67%), while the lowest was observed in muscle samples (36.67%) with intermediate prevalence in other samples like heart, liver, and water samples of those places (Table 1). Fecal contamination, poor hygienic standards in slaughterhouses, and utilization of contaminated water might be responsible factors for the higher prevalence of *Klebsiella* spp. obtained in chevon samples in the present study, as opined by Singh *et al*. (1997). The 56.67% prevalence of *Klebsiella* spp. found in chevon in

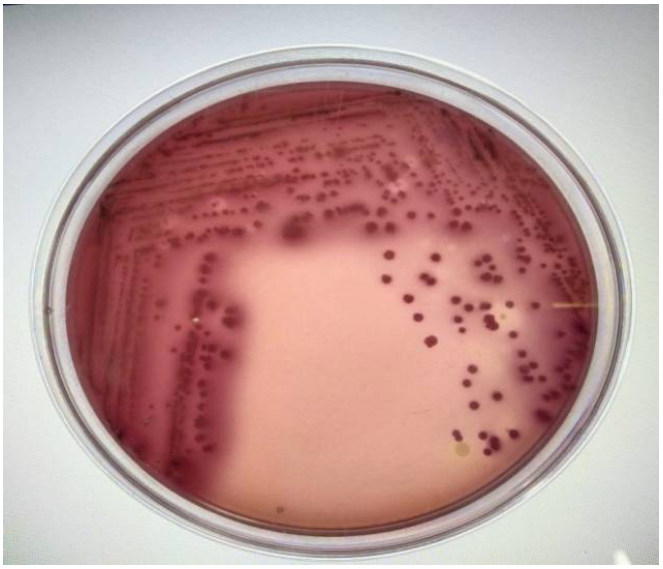

Fig. 1: Pink colored mucoid dome-shaped colonies of *Klebsiella* spp. on MacConkey agar

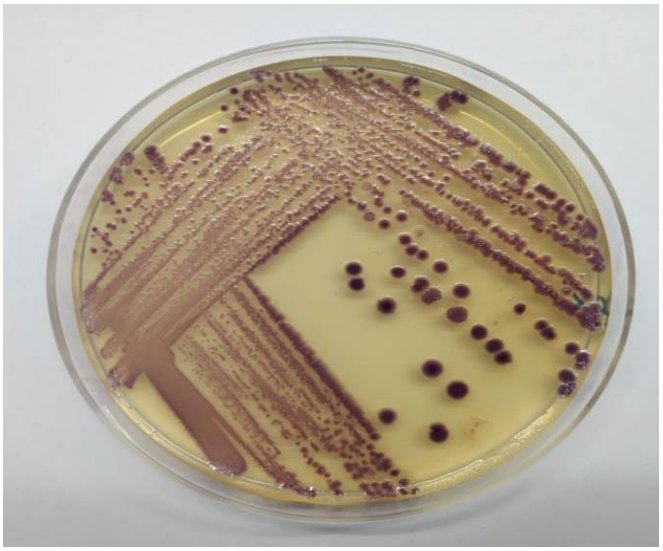

Fig. 2: Purple-Magenta colored mucoid colonies of *Klebsiella* spp. on *Klebsiella* selective agar

the present study was higher than the earlier finding of 34.0% by Singh and Sharma (2001), while Chuku *et al.* (2016) reported a higher prevalence rate up to 82.5% of *Klebsiella* spp.

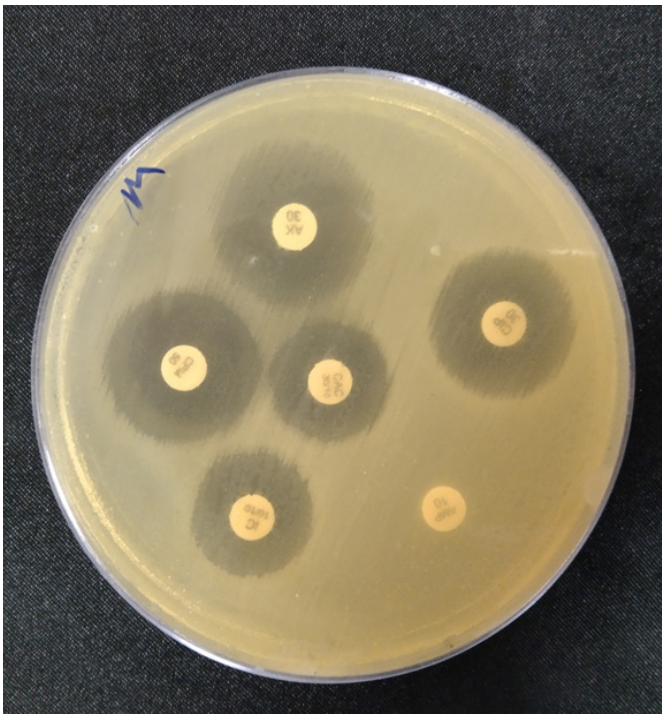

Fig. 3a: *In vitro* antimicrobial sensitivity test of *Klebsiella* spp. Isolates: CIP- Ciprofloxacin, AMP–Ampicillin, AK–Amikacin, CPM–Cefepime, CAC– Ceftazidime/Clavulinic acid

Table 1: Recovery of *Klebsiella* spp. isolates from different samples

Types of samples	*No. of samples collected*	*No. of positive samples*	*Prevalence (%)*
Muscles	30	11	36.67
Intestine	30	23	76.67
Liver	30	20	66.67
Heart	30	14	46.67
Water	30	17	56.67
Total	150	85	56.67

Antibiotic sensitivity Pattern of *Klebsiella* spp. Isolates

All the genotypically confirmed *Klebsiella* spp. (n = 85) isolates were tested for antibiotic sensitivity against ten different antibiotics (Figs. 3a and b). The antibiogram of all isolates revealed a high degree of sensitivity to amoxicillin/clavulanic acid (89.41%) and gentamicin (70.58%), while they showed complete resistance towards ampicillin and ceftazidime (100.00% each). The resistance was also observed towards ciprofloxacin, ceftriaxone and cefepime to the extent of 70.58%, 67.05% and 75.29%, respectively. In the case of imipenem and amikacin, only 29.41% and 12.94% of isolates showed resistance, while most of the isolates showed intermediate sensitivity to them (Table 2). Our results were in close association with the findings of Singh and Sharma (2001) and Anning *et al.* (2019), who revealed that 100% isolates were resistant to ampicillin and 98.00% isolates showed susceptibility to gentamicin.

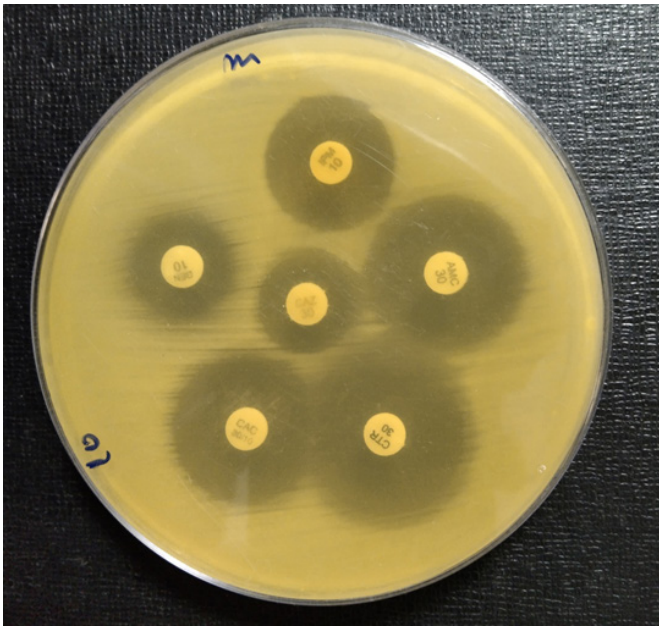

Fig 3b: *In vitro* antimicrobial sensitivity test of *Klebsiella* spp. Isolates: GEN-Gentamicin, CTR–Ceftriaxone, AMC– Amoxicillin/Clavulinic acid), CAZ– Ceftazidime), CAC– Ceftazidime/Clavulinic acid, IPM–Imipenem

Table 2: *In vitro* anti-microbial drug resistance pattern of *Klebsiella* spp. isolates (n=85)

Sr. No.	*Anti-microbial agents*	*Sensitive*	*Intermediate*	*Resistant*
1.	Gentamicin	60 (70.58)	0	25 (29.41)
2.	Amikacin	52 (61.17)	22 (25.88)	11 (12.94)
3.	Ampicillin	0	0 (0.0)	85(100.0)
4.	Imipenem	30 (35.29)	30 (35.29)	25 (29.41)
5.	Ciprofloxacin	11 (12.94)	14 (16.47)	60 (70.58)
6.	Ceftazidime	0	0	85(100.0)
7.	Ceftazidime/clavulinic acid	0	22 (25.88)	63 (74.11)
8.	Ceftriaxone	22 (25.88)	6 (07.08)	57 (67.05)
9.	Cefepime	1 (01.17)	20 (23.52)	64 (75.29)
10.	Amoxicillin/clavulanic acid	76 (89.41)	9 (10.58)	0

Figures in the parentheses indicate percent values

Conclusion

Isolation of multiple drug-resistant strains from meat is of public health significance due to the fact that in maximum outbreaks of Klebsiellosis, foods of animal origin have been implicated. The prevalence of resistant *Klebsiella* spp. was observed in 85 out of 150 (56.67%) chevon samples studied. Multidrug resistance was prevalent in *Klebsiella* spp. indicating the presence of multiple-drug resistance genes on a similar mobile genetic element. The possibility of minimizing resistance by controlling the use of antibiotics is a rational approach. Tracking and monitoring the spread of ESBL producing strains in food-producing animals is needed to improve and safeguard public health.

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