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Multidrug resistant-*Proteus mirabilis* and *Proteus vulgaris* isolated from milk and meat samples in Tirupati, Andhra Pradesh: An emerging public health threat

Sowjanya Priya Ronanki, P Ramya, A Jagadeesh Babu and B Sreedevi

Abstract
Proteus is an emerging food borne pathogen having public health significance. *P. mirabilis* and *P. vulgaris* are zoonotic human pathogens causes urinary tract infection (UTI), nosocomial infection, wound infection and septicaemia, therefore, a potential threat to public health. The present study was undertaken to detect multi-drug resistant bacteria – *P. mirabilis* and *P. vulgaris* from raw milk and meat samples procured for about 4 weeks from 50 different wards of Tirupati, Andhra Pradesh. All the isolates were subjected to 14 antibiotic discs by using standard disk diffusion method. All the isolates of *P. mirabilis* from milk and meat samples have shown 100% resistance to Tetracycline, Colistin, Erythromycin and Penicillin-G. All the *P. vulgaris* isolates from milk have shown 100% resistance to Tetracycline, Colistin, Erythromycin, Penicillin-G, Ampicillin and Polymyxin-B. *P. vulgaris* isolates from meat have shown 100% resistance towards Tetracycline, Colistin, Erythromycin, Penicillin-G, Ampicillin and Vancomycin. All the isolates of *P. mirabilis* and *P. vulgaris* have shown sensitivity to Gentamicin.

Keywords: Meat, milk, *Proteus mirabilis*, *Proteus vulgaris*, multi drug resistant, disc diffusion method

1. Introduction
Proteus rods are opportunistic bacterial pathogens which under favourable conditions cause urinary tract infections (UTIs), wound infections, meningitis in neonates or infants and rheumatoid arthritis (O'hara *et al.*, 2000; Janda & Abbot, 2006) [20, 9].
Both *P. mirabilis* and *P. vulgaris* are widely distributed in the environment and have been isolated from the intestinal tract of mammals, birds and reptiles. *P. mirabilis*, and to a lesser extent *P. vulgaris* are common inhabitants of the human gastrointestinal tract. *P. mirabilis* in particular, may also colonize the urinary tract under certain circumstances, an opportunistic pathogen and one of the principal causes of urinary tract infections (UTIs) in hospitalised patients with indwelling urinary catheters, whereas, *Proteus vulgaris* is less commonly with UTIs (Manos and Belas, 2006) [16].
Epidemiological investigations indicated that meat products, bean products, fish and cold dishes are commonly associated with food poisoning related to *P. mirabilis* (Wang *et al.*, 2010; Cao *et al.*, 2011; Jiang *et al.*, 2017) [34, 4, 10]. Hence, *P. mirabilis* may pose a relatively great threat to food safety and public health.
There are now multiple resistant forms of this bacterium which indicates a major food issue (Lei *et al.*, 2016) [14]. The antibiotic resistance of this pathogen is a significant public health concern. The indiscriminate use of antibiotics for therapeutic purposes in animals and humans led to the development of antibiotic resistance in the organism. Drug resistant strains of this organism were isolated from clinical and food samples (Nahar *et al.*, 2014; Wong *et al.*, 2013 and Kim *et al.*, 2005) [17, 15, 13].
The problem of antibiotic resistance in microorganisms is mainly due to the natural resistance of definite species to certain antibiotics, the transfer of antibiotic resistance between the species and the use of sub-therapeutic doses of antibiotics in animal feeds to improve animal productivity, routine use of antimicrobial agents for domestic livestock to prevent and treat diseases, contributes to the emergence of antibiotic resistant bacteria that can be subsequently transferred to humans through the food chain (Osibote *et al.*, 2014; Iroha *et al.*, 2011; kim *et al.*, 2005) [22, 8, 13]. This will be largely responsible for the emergence of drug resistance bacteria in animal products such as meat and milk products.
Resistance to multiple antimicrobials has been documented in *Proteus* spp. from the livestock.

The emergence of drug-resistant *Proteus* spp. that could potentially be transmitted to humans through contaminated food has been found as a potential public health threat to humans (Seiffert *et al*., 2013; Wong *et al*., 2013) [29, 35].

Impacts of the study

- Emerging food borne pathogens like *Proteus species* are of major public health concern and are responsible for causing outbreaks of food poisonings and transfer of multidrug resistant bacteria to the human beings.
- This study has shown the high prevalence of multi drug resistant *Proteus species* in milk and meat samples. The overall proportion of antimicrobial resistance was high. As a result, this study suggests that there is a risk of dissemination of resistance *Proteus spp*. through the food chain. This will lead to the transfer of the drug resistant bacteria to the human beings through the consumption of milk and meat products.
- Considering the high rate of raw milk and raw meat contamination with the *Proteus* bacteria, sanitary practice during collecting, transporting and handling is recommended, since the consumption of contaminated milk and meat may inflict an important public health risk.

2. Materials and Methods

2.1 Collection and processing of milk and meat samples

394 Meat and 347 milk samples were collected from retail meat shops, local milk vendors, milk booths, cattle farms of all municipal wards in Tirupati. Sterile polythene zip lock bags and sterile sample collection bottles were used for collection of meat and milk samples respectively. Each bag and bottle were labelled with sample number and particulars about samples. Within 2 hours, all the samples were transferred in an ice box to the laboratory of Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, Tirupati for further processing. All the procedures were carried out in a biosafety cabinet with due precautions.

Identification of all the isolates to the genus / species level was performed using conventional cultural and biochemical techniques as described by Al-Hamdani and Al-Hashimy (2020) [37], Naidu *et al.*, (2020) [18], Nahar *et al.*, (2014) [17], Ali and Jasim, (2014) [2]. All the biochemically confirmed *Proteus* isolates were further subjected to PCR by targeting genus and species-specific genes.

2.2 *In-vitro* antibiotic sensitivity testing by disc diffusion method

All the *Proteus* isolates were tested by Disc Diffusion (DD) method to detect their antibiotic sensitivity pattern. This method was performed using Mueller Hinton agar by Kirby-Bauer method with the following antibiotic discs: Gentamicin (GEN) 10 µg, Ampicillin (AMP) 30 µg, Ciprofloxacin (CIP) 5 µg, Ceftazidime (CAZ) 30 µg, Ceftriaxone (CTR) 30 µg, Trimethoprim (Tr) 30 µg, Colistin (CL) 25 µg, Penicillin-G (P) 10U, Tetracycline (TE) 30 µg, Ofloxacin (OF) 5 µg, Erythromycin (E) 15 µg, Chloramphenicol (C) 30 µg, Vancomycin (VA) 30 µg, Polymyxin-B (PB) 50U.

Bacterial suspension was made by transferring 4-5 colonies from primary isolated medium that is XLD agar, Nutrient agar to 5 ml of nutrient broth by touching the top of the colonies with a flame sterilized and cooled platinum loop. The resulting culture after incubation at 37 °C for 8 hours was compared with the turbidity standard prepared separately for adjustment of bacterial suspension. About 200µl of each inoculum was seeded on Muller Hinton agar plates and evenly spread over the entire surface of the Mueller Hinton agar plates. The plates were then allowed to dry for 3-5 mins and antibiotic-impregnated discs were then applied to the surface of the inoculated plates with sterile forceps. Each disc was gently pressed down onto the agar to ensure complete contact with the agar surface. A minimum distance of 24 mm between the discs was maintained and the plates were incubated at 37 °C and examined after 24 hrs. The diameter of the zone of complete inhibition was measured and interpreted as per CLSI (2018) guidelines (M45-A2) (Table-1) (Fig1&2).

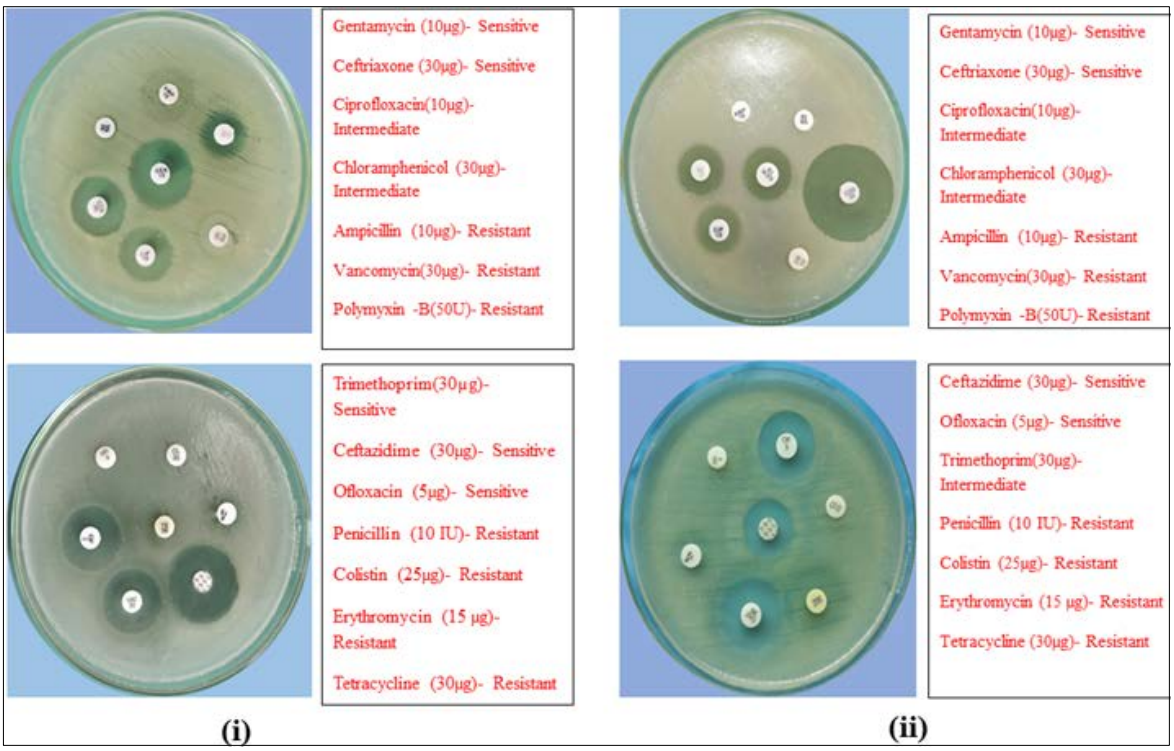

Fig 1: i) Plate showing anti-bio gram of *P. mirabilis*, ii) Plate showing anti-bio gram *P. vulgaris*

3. Results

The overall prevalence of *Proteus* species in 50 different wards of Tirupati, Andhra Pradesh was 40.1% (158 positive samples, n = 394) in meat samples and 5.19% (18 positive samples, n =347) in the milk samples. Among the 176 molecularly confirmed *Proteus* isolates, 18 (10.23%) were from milk and 158 (89.77%) were from meat. The results confirmed that out of 139 biochemically confirmed isolates of *P. mirabilis* targeted for *ure R* gene 132 (94.96%) isolates carried the gene *ure R* and among the 132 isolates,13 (9.85%) were from milk and 119 (90.15%) from meat. In addition, out of 50 isolates of biochemically confirmed *P. vulgaris* targeted for *urease C* gene, 44 (88.0%) isolates have shown the presence of *urease C* gene. Among the 44 molecularly confirmed isolates of *P. vulgaris*, 5 (11.36%) were from milk samples and 39 (88.64%) were from meat samples.

3.1 Anti-bio gram of *P. mirabilis* isolated from milk

The *P. mirabilis* isolates from milk have shown maximum sensitivity to Gentamycin (53.86%) followed by Ceftazidime (53.85%), Ceftriaxone (53.85%), Chloramphenicol (30.77%), Trimethoprim (30.77%) Ciprofloxacin (23.08%), Ofloxacin (23.08%), Vancomycin (7.69%) and Polymyxin-B (7.69%) whereas maximum resistance was observed towards Tetracycline (100%), Colistin (100%), Erythromycin (100%), Penicillin-G (100%), followed by Ampicillin (92.31%), Vancomycin (92.31%), Polymyxin-B (92.31%), Ciprofloxacin (76.92%), Trimethoprim (69.23%), Ofloxacin (61.54%), Chloramphenicol (53.86%), Ceftriaxone (46.15%), Gentamycin (30.77%) and Ceftazidime (23.077%).

3.2 Anti-bio gram of *P. mirabilis* isolated from meat

The *P. mirabilis* isolates from meat have shown maximum sensitivity to Gentamycin (68.91%) followed by Ceftriaxone (50.42%), Ceftazidime (42.02%), Ciprofloxacin (23.53%), Trimethoprim (20.17%), Ofloxacin (15.97%), Chloramphenicol (14.28%), Vancomycin (14.28%) and Polymyxin-B (14.28%) whereas the isolates have shown maximum resistance to Tetracycline (100%), Colistin(100%), Erythromycin (100%), Penicillin-G (100%), followed by Ampicillin(99.16%), Vancomycin(94.12%), Polymyxin-B (91.60%), Trimethoprim (76.47%), Ofloxacin (68.91%), Chloramphenicol (64.71%), Ciprofloxacin(59.66%), Gentamycin (30.25%) Ceftriaxone (26.15%) and Ceftazidime (24.37%)

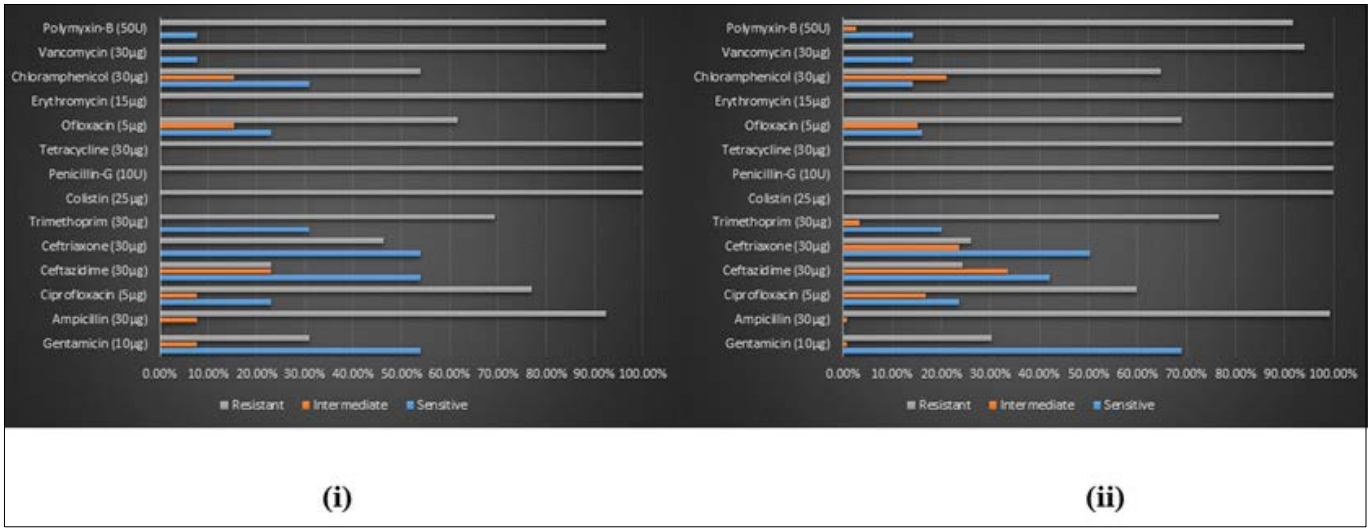

Fig 2: i) Anti-bio gram patteras of positive *P. mirabilis* isolates from milk samples, ii) Anti-bio gram patteras of positive *P. mirabilis* isolates from meat samples

3.3 Anti-bio gram of *P. vulgaris* isolated from milk

The *P. vulgaris* isolates from milk have shown maximum sensitivity towards Gentamycin (80%) followed by Chloramphenicol (40%), Ceftazidime (20%), Ciprofloxacin (20%), Ofloxacin (20%) and Vancomycin (20%) whereas the isolates have shown maximum resistance to Tetracycline (100%), Colistin (100%), Erythromycin (100%) Penicillin-G (100%), Ampicillin (100%), and Polymyxin-B (100%), followed by Vancomycin (80%), Trimethoprim (80%), Ofloxacin (80%), Ceftriaxone (60%), Ciprofloxacin (40%), Chloramphenicol (40%), Gentamycin (20%) and Ceftazidime (20%).

3.4 Anti- bio gram of *P. vulgaris* isolated from meat

The *P. vulgaris* isolates from meat have shown maximum sensitivity towards Ceftriaxone (46.15%), followed by Ceftazidime (35.90%), Gentamycin (30.77%), Ciprofloxacin (12.82%), Trimethoprim (10.26%), Ofloxacin (5.13%) and Chloramphenicol (2.56%) and the isolates have shown maximum resistance towards Tetracycline(100%), Colistin (100%), Erythromycin (100%), Penicillin-G (100%), Ampicillin (100%), Vancomycin (100%) followed by Polymyxin-B (97.44%), Ofloxacin (92.31%), Trimethoprim (87.18%), Chloramphenicol (82.05%), Ciprofloxacin (71.79%), Gentamycin (69.23%) Ceftriaxone (41.03%) and Ceftazidime 23.08%).

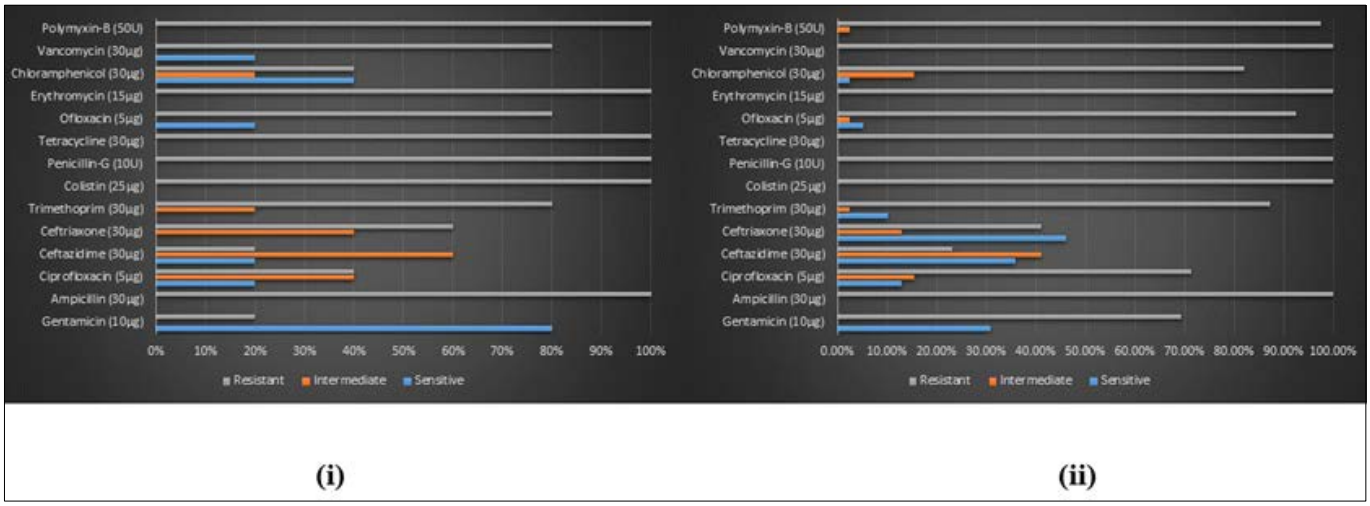

Fig 3: i) Anti- bio gram patterus of positive *P. vulgaris* isolates from milk samples, ii) Anti- bio gram patterus of positive *P. vulgaris* isolates from meat samples

4. Discussion

Antibiotic sensitivity test was performed for all 176 *Proteus* isolates to detect the sensitivity/resistance pattern by Kirby-Bauer's disc diffusion method using 14 most commonly used and commercially available antibiotic discs.

4.1 Antimicrobial resistance profile of *P. mirabilis* and *P. vulgaris* in milk

In the present study, most of the *P. mirabilis* isolates from raw milk samples were highly sensitive to entamycin (53.86%) followed by ceftriaxone (53.85%), ceftazidime (53.85%), chloramphenicol (30.77%). The sensitivity of gentamycin (53.86%) in the current study was in agreement with the findings of Reta *et al*., (2016) [25]; Rudenko *et al*., (2021) [26]; Kaur *et al*., (2015) [12]; Sahoo *et al*., (2020) [27] who have reported a sensitivity of about 33.3%, 64.5%, 91%, 95% to gentamicin respectively. The Sensitivity of ceftazidime (53.85%) was found in contrast with the findings of Olunrebi *et al*., (2021) [21], Goyal *et al*., (2009) [6] who have reported 11.2% and 100% respectively. In the present study *P. mirabilis* was reported to be 53.85% sensitive to Ceftriaxone which was in contrast with the findings of Sahoo *et al*., (2020) [27] who have reported 82.23% of sensitivity. The sensitivity of chloramphenicol (30.77%) in the current study was in agreement with the results of Reta *et al*., (2016) [25] who have reported 33.3% sensitivity and Sumati *et al*., (2008) [31] who have reported 75% sensitivity to chloramphenicol.

In the present study all of the *P. mirabilis* isolates from raw milk samples have shown multidrug resistance to tetracycline (100%), colistin (100%), erythromycin (100%), penicillin (100%) followed by ampicillin (92.31%), vancomycin (92.31%), polymyxin-B (92.31%), ciprofloxacin (76.92%), trimethoprim (69.23%). The results were in agreement with the findings of Tartor *et al*., (2021) [33], who have reported 90.9% resistance in tetracycline, Kaur *et al*., (2015) [12] who have reported 83% and 86% resistance in colistin and penicillin respectively, Olunrebi *et al*., (2021) [21] reported 71.74% resistance in erythromycin. Ampicillin resistance (92.31%) was in agreement with the reports of Younis *et al*., (2017) [36]; Kaur *et al*., (2015) [12]; Reta *et al*., (2016) [25] who have reported 100%, 62.51%, 55.6% respectively. The resistance to vancomycin (92.31%) and trimethoprim (69.23%) were nearly similar to the results of Younis *et al*., (2017) [36] who have reported 68.6% and 74.3% respectively. The resistance of ciprofloxacin (76.92%) was in agreement with the results of Tartor *et al*., (2021) [33] who have reported 60.60% resistance in ciprofloxacin.

In the present study, most of the *P. vulgaris* isolates from raw milk samples in the current study were highly sensitive to Gentamycin (80%) followed by Chloramphenicol (40%), Ceftazidime (20%), Ciprofloxacin (20%), Ofloxacin (20%) and Vancomycin (20%).

P. vulgaris was reported to be 80% sensitive to gentamycin which was similar to that of the work reported by Olunrebi *et al.* (2021) [21]; Sahoo *et al.* (2020) [27]; Kaur *et al.* (2015) [12] who have reported a sensitivity of 94.34%,95.52%,91% respectively. The present study reported 40% sensitivity towards chloramphenicol which was in agreement with the reports of Younis *et al.* (2017) [36] and Reta *et al.* (2016) [25], who have reported a similar 37.1% and 33.3%sensitivity respectively. In contrast 80.85% sensitivity was observed towards chloramphenicol by Olunrebi *et al.* (2021) [21]. In the present study *P. vulgaris* was reported to be 20% sensitive to ceftazidime which was in contrast to the results obtained by Olunrebi *et al.* (2021) [21] have reported a 100% resistance.

In the present study, all of the *P. vulgaris* isolates from raw milk samples have shown maximum resistance to Tetracycline (100%), Colistin (100%), Erythromycin (100%) Penicillin-G (100%), Ampicillin (100%), and Polymyxin-B (100%), followed by Vancomycin (80%), Trimethoprim (80%), Ofloxacin (80%) and Ceftriaxone (60%),

Resistance to colistin, erythromycin, ampicillin in the present study was 100% which was in agreement with the results reported by Kateete *et al*., (2013) [11] who have reported 100% resistance in colistin, erythromycin, ampicillin. In the present study, resistance of *P. vulgaris* towards penicillin-G (100%) was in contrast with the reports of Sahoo *et al*., (2020) [27] and Sumati *et al*., (2008) [31] who have reported 8.66% and 42.9% sensitivity. Resistance of tetracycline (100%) in the present study was found in contrast with the results obtained by Sahoo *et al*., (2020) [27] and Olunrebi *et al*., (2021) [2] who have reported 51.31 and 71.4% resistance. In the present study, resistance of vancomycin (80%) and trimethoprim (80%) was in agreement with the results obtained by Younis *et al*., (2017) [36] who have reported 68.6% and 74.3%resistance respectively.

4.2 Antimicrobial resistance profile of *P. mirabilis* and *P. vulgaris* in meat

All the 119 *P. mirabilis* isolates and 39 *P. vulgaris* isolates

from meat were subjected to antibiotic sensitivity test using 14 different and most commonly used antibiotics in veterinary medicine

In the present study, most of the *P. mirabilis* isolates from raw meat samples in the current study were highly sensitive to gentamycin (68.91%) followed by ceftriaxone (50.42%), ceftazidime (42.02%) and ciprofloxacin (23.53%).

Higher sensitivity of the *P. mirabilis* isolates to gentamicin (68.91%) from meat samples in the present study was almost similar with the findings of Nahar *et al.,* (2014) [17] and Wong *et al.,* (2013) [15] who have reported 41.6% and 38% sensitivity, respectively. The sensitivity of ceftriaxone (50.42%) in the present study was found in contrast with the results of Ogunleye *et al.,* (2016) [19] who have reported 9.1% sensitivity and Wong *et al.,* (2013) [15] who have reported 36% resistance to ceftriaxone. In the present study, *P. mirabilis* isolates were reported to be 50.42% sensitive to ceftazidime which was in contrast with the results of Algammal *et al.,* (2021) [1], Naidu *et al.,* (2020) [18] and Goyal *et al.,* (2009) [6] who have reported 14.3%, 11.4% and 100% sensitivity towards ceftazidime respectively. The sensitivity of ciprofloxacin (23.53%)in the present study was found in contrast with the results of Nahar *et al.,* (2014) [17] who have reported 50% sensitivity and Wong *et al.,* (2013) [15] who have reported 52% resistance to ciprofloxacin.

In the present study, all of the *P. mirabilis* isolates from raw meat samples have shown maximum resistance to Tetracycline (100%), Colistin (100%), Erythromycin (100%), Penicillin-G (100%), followed by Ampicillin (99.16%), Vancomycin (94.12%), Polymyxin-B (91.60%), Trimethoprim (76.47%), Ofloxacin (68.91%), Chloramphenicol (64.71%).

Multi-drug resistance in erythromycin (100%), colistin (100%), tetracycline (100%), penicillin-G (100%)was found and these findings were similar to the findings of Algammal *et al.,* (2021) [1] who have reported 100% resistance in erythromycin and penicillin-G, Pattanayak *et al.,(*2018) [4] who have reported 100% resistance to erythromycin and tetracycline, Dadheech *et al.,* (2015), Wong *et al.,* (2013), Salih *et al.,* (2019) [28] and Kim *et al.,* (2005) [17] who have reported 100% resistance in tetracycline, Shelenkov *et al.,* (2020) [30] who reported 100% resistance in colistin.

In the present study, resistance towards ampicillin was 99.16% which was in correlation with the results of Kim *et al.,* (2005) [17] and Gupta *et al.,* (2014) [7] who have reported 83% and 100% resistance respectively. The resistance towards polymyxin-B was 91.60% which was in correlation with the results of Pattanayak *et al.,* (2018) [4] who have reported 100% resistance. The resistance exhibited to chloramphenicol (64.71%) in the study was found to be similar to the findings of Wong *et al.,* (2013) [15] who have reported 66% of resistance to chloramphenicol and found contrast with the results of Gupta *et al.,* (2014) [7] and Naidu *et al.,* (2020) [18] who have reported 100% and 26.22% resistance respectively.

In the present study, most of the *P. vulgaris* isolates from raw meat samples have shown maximum sensitivity towards ceftriaxone (46.15%), followed by ceftazidime (35.90%) and Gentamycin (30.77%).

In the present study, *P. vulgaris* was reported to be 46.15% sensitive to ceftrioxane and 35.90% sensitive to ceftazidime which was in agreement with the results of Sun *et al.,* (2020) who have reported that the *P. vulgaris* isolates in their study was found to be sensitive to ceftrioxane and ceftazidime and in contrast Ogunleye *et al.,* (2016) [19] who have reported resistance to ceftrioxane and ceftazidime.

Owoseni *et al.* (2021) [23] reported 18.9% and 13.5% resistance towards gentamycin and ciprofloxacin which were found more or less similar to the findings in the current study reporting 18.9% and 13.5% sensitivity to gentamycin and ciprofloxacin respectively.

In the present study, most of the *P. vulgaris* isolates from raw meat samples have shown maximum resistance towards tetracycline (100%), Colistin (100%), erythromycin (100%), penicillin-g (100%), ampicillin (100%), Vancomycin (100%) followed by polymyxin-b (97.44%), ofloxacin (92.31%), trimethoprim (87.18%), chloramphenicol (82.05%), ciprofloxacin (71.79%).

Mandal *et al.,* (2015) [15] reported 100%, 100%,90%,80% resistance pattern of *P. vulgaris* isolates towards penicillin, ampicillin, erythromycin, tetracycline respectively which were almost similar with the present study showing 100% resistance to penicillin, ampicillin, erythromycin, tetracycline. Owoseni *et al.,* (2021) [23] reported 10.8% susceptibility towards ofloxacin which was in contrast with the present study which was shown 92.31% resistance to ofloxacin. In the present study, the resistance exhibited to ciprofloxacin was 68.9% which was found to be similar with the findings of Salih *et al.,* (2019) [28] who have reported 68.9% resistance to ciprofloxacin.

Table 1: Interpretation chart for ABST as per CLSI (2018) guidelines

S. No.	Antimicrobial agent	Symbol	Disc Conc.	Interpretative Criteria		
				Resistant Mm (≤)	Intermediate	Sensitive Mm (≥)
1	Gentamicin	GEN	10 µg	12	13-14	15
2	Ampicillin	AMP	30 µg	13 mm	14-16 mm	17 mm
3	Ciprofloxacin	CIP	5 µg	21	22-25	26
4	Ceftazidime	CAZ	30 µg	17	18-20	21
5	Ceftriaxone	CTR	30 µg	19	20-22	23
6	Trimethoprim	Tr	30 µg	10	11-15	16
7	Colistin	CL	25 µg	-	-	11-15
8	Penicillin-G	P	10U	14	-	15
9	Tetracycline	TE	30 µg	11	12-14	15
10	Ofloxacin	OF	5 µg	12	13-15	16
11	Erythromycin	E	15 µg	15	16-20	21
12	Chloramphenicol	C	30 µg	12	13-17	18
13	Vancomycin	VA	30 µg	14	15-16	17
14	Polymyxin-B	PB	50U	-	-	12-16

Table 2: Antibiotic resistance patterns of positive *P. mirabilis* isolates from milk samples

S. No.	Antimicrobial agent	Sensitive	Intermediate	Resistant
1	Gentamicin (10 µg)	7 (53.86%)	2 (15.38%)	4 (30.77%)
2	Ampicillin (30 µg)	0	1 (7.69%)	12 (92.31%)
3	Ciprofloxacin (5 µg)	3 (23.08%)	0	10 (76.92%)
4	Ceftazidime (30 µg)	7 (53.85%)	3 (23.08%)	3 (23.08%)
5	Ceftriaxone (30 µg)	7 (53.85%)	0	6 (46.15%)
6	Trimethoprim (30 µg)	4 (30.77%)	0	9 (69.23%)
7	Colistin (25 µg)	0	0	13 (100%)
8	Penicillin-G (10U)	0	0	13 (100%)
9	Tetracycline (30 µg)	0	0	13 (100%)
10	Ofloxacin (5 µg)	3 (23.08%)	2 (15.38%)	8 (61.54%)
11	Erythromycin (15 µg)	0	0	13 (100%)
12	Chloramphenicol (30 µg)	4 (30.77%)	2 (15.38%)	7 (53.85%)
13	Vancomycin (30 µg)	1 (7.69%)	0	12 (92.31%)
14	Polymyxin-B (50U)	1 (7.69%)	0	12 (92.31%)

Table 3: Antibiotic resistance patterns of positive *P. mirabilis* isolates from meat samples

S. No.	Antimicrobial agent	Sensitive	Intermediate	Resistant
1	Gentamicin (10 µg)	82(68.91%)	1 (0.84%)	36 (30.25%)
2	Ampicillin (30 µg)	0	1 (0.84%)	118 (99.16%)
3	Ciprofloxacin (5 µg)	28 (23.53%)	20 (16.81%)	71 (59.66%)
4	Ceftazidime (30 µg)	50 (42.02%)	40 (33.61%)	29 (24.37%)
5	Ceftriaxone (30 µg)	60 (50.42%)	28 (23.53%)	31 (26.05%)
6	Trimethoprim (30 µg)	24 (20.17%)	4 (3.36%)	91 (76.47%)
7	Colistin (25 µg)	0	0	119 (100%)
8	Penicillin-G (10U)	0	0	119 (100%)
9	Tetracycline (30 µg)	0	0	119 (100%)
10	Ofloxacin (5 µg)	19 (15.97%)	18 (15.13%)	82 (68.91%)
11	Erythromycin (15 µg)	0	0	119 (100%)
12	Chloramphenicol (30 µg)	17 (14.28%)	25 (21%)	77 (64.71%)
13	Vancomycin (30 µg)	7 (14.28%)	0	112 (94.12%)
14	Polymyxin-B (50U)	7 (14.28%)	3 (2.52%)	109 (91.60%)

Table 4: Antibiotic resistance patterns of positive *P. vulgaris* isolates from milk samples

S. No.	Antimicrobial agent	Sensitive	Intermediate	Resistant
1	Gentamicin (10 µg)	4 (80%)	0	1 (20%)
2	Ampicillin (30 µg)	0	0	5 (100%)
3	Ciprofloxacin (5 µg)	1 (20%)	2(40%)	2 (40%)
4	Ceftazidime (30 µg)	1 (20%)	3(60%)	1 (20%)
5	Ceftriaxone (30 µg)	0	2(40%)	3 (60%)
6	Trimethoprim (30 µg)	0	1(20%)	4 (80%)
7	Colistin (25 µg)	0	0	5 (100%)
8	Penicillin-G (10U)	0	0	5 (100%)
9	Tetracycline (30 µg)	0	0	5 (100%)
10	Ofloxacin (5 µg)	1 (20%)	0	4 (80%)
11	Erythromycin (15 µg)	0	0	5 (100%)
12	Chloramphenicol (30 µg)	2 (40%)	1(20%)	2 (40%)
13	Vancomycin (30 µg)	1 (20%)	0	4 (80%)
14	Polymyxin-B (50U)	0	0	5 (100%)

Table 5: Antibiotic resistance patterns of positive *P. vulgaris* isolates from meat samples

S. No.	Antimicrobial agent	Sensitive	Intermediate	Resistant
1	Gentamicin (10 µg)	12 (30.77%)	0	27 (69.23%)
2	Ampicillin (30 µg)	0	0	39 (100%)
3	Ciprofloxacin (5 µg)	5 (12.82%)	6 (15.38%)	28 (71.19%)
4	Ceftazidime (30 µg)	14 (35.90%)	16 (41.02%)	9 (23.08%)
5	Ceftriaxone (30 µg)	18 (46.15%)	5 (12.82%)	16 (41.03%)
6	Trimethoprim (30 µg)	4 (10.26%)	1 (2.56%)	34 (87.18%)
7	Colistin (25 µg)	0	0	39 (100%)
8	Penicillin-G (10U)	0	0	39 (100%)
9	Tetracycline (30 µg)	0	0	39 (100%)
10	Ofloxacin (5 µg)	2 (5.13%)	1 (2.56%)	36 (92.31%)
11	Erythromycin (15 µg)	0	0	39 (100%)
12	Chloramphenicol (30 µg)	1 (2.56%)	6 (15.38%)	32 (82.05%)
13	Vancomycin (30 µg)	0	0	39 (100%)
14	Polymyxin-B (50U)	0	1 (2.56%)	38 (97.44%)

5. Conclusion

Our findings indicate the existence of high drug resistant bacteria in milk and meat samples. The high use of β-lactam antibiotics and cephalosporins and inappropriate infection control procedures in the hospitals might be the cause of rising rates of resistance among these bacteria. Moreover, longer duration of prophylactic antimicrobial exposure may contribute to organisms for developing resistance. Nowadays, antimicrobial resistance is a global problem. It is a drain on the global economy due to lengthen the therapeutic period and higher costs of treatment. The goal of the global action plan is to ensure successful treatment with effective and safe antimicrobials that are specific and sensitive.

This study have shown the high prevalence of multi drug resistant *Proteus species* in milk and meat samples. The overall proportion of antimicrobial resistance was high. As a result, this study suggests that the risk of dissemination of resistance *Proteus spp*. through the food chain. This will lead to the transfer of the drug resistant bacteria to the human beings through the consumption of milk and meat products. Considering the high rate of raw milk and raw meat contamination with the *Proteus* bacteria, sanitary practice during collecting, transporting and handling is recommended since the consumption of contaminated milk and meat may inflict an important public health risk. Farmers and butchers are need to be trained on improved control of contamination of milk and meat through adoption of good hygienic practices during collecting, transporting and vending of milk and meat.

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7. Conflicts of interest

The authors have no conflicts of interest to declare. All the co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report.

8. Ethical committee approval

The ethical committee approval is not needed for the current study as it was done by collecting the meat samples from meat shops which are kept for sale and the milk samples were collected from local milk vendors and farms. In this study no invasive procedure was used on animals and no animal was harmed in the study.

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