Original Article

Isolation and Characterization of *Acinetobacter baumannii* from Chicken Meat Samples in North India

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ABSTRACT

Meat is consumed as food and rich source of nutrients in many parts of the world. Due to poor hand hygiene and other sources, meat samples get contaminated with many bacterial species. Most of the pathogenic bacteria are often traced in hospital settings but some of them in the recent years have been recovered from outside hospital environment like; *Acinetobacter baumannii*, *Salmonella*, *E. coli, Clostridium, Staphyloccus, Klebsiella,* and *Pseudomonas* species. The aim of the study was to investigation of bacterial load, types and their resistance profile in the chicken meat collected from various slaughterhouses of North India. A total of 50 samples (meat, surface swabs and knife swabs) were processed in the microbiology laboratory. CFUs were counted by Spread Plate Count (SPC) method and cultured bacteria were analyzed by MALDI-TOF MS. Antimicrobial susceptibility testing was performed using Kirby-Bauer disk diffusion assay as per CLSI guidelines. We isolated and characterized the potential pathogenic bacteria like; *E. coli* (5), *Acinetobacter baumannii* (4), *Enterobacter cloacae* (2), *Pseudomonas aeruginosa* (1) and *Providencia stuartii* (1) from raw meat, knifen and surface samples from the retail meat shop in Mullana territory. Although a number of pathogenic strains were isolated from meat samples, a low resistance was reported for all the isolates recovered from meat samples, comparative to that of clinical Isolates in the region.

Key words: Chicken Meat, Food Poisoning, *Acinetobacter baumannii, E. coli*, MALDI-TOF MS.

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INTRODUCTION

Bacteria contaminating meat samples may be carriers or opportunistic pathogens of clinical relevance. They might harbor significant resistance genes, which confer antimicrobials resistance in both humans and animals. Meat and dairy products are considered as potential vectors for the transfer of multidrug-resistant (MDR) bacteria between animals and humans.[1] All the ESKAPE pathogens (*Acinetobacter baumannii, Enterococcus* species, *Enterobacter* species, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) cause the most of the serious nosocomial infections in hospital settings among seriously ill patients with compromised immune system all over the globe.[2] However, most of the ESKAPE pathogens are naturally found as commensal or colonizers of humans and animals and do not cause harm to the host.[3] Interestingly, *Pseudomonas aeruginosa* and *Acinetobacter* species have commonly been investigated in the soil, aquatic environments, and might be an indication of dissemination of contamination from animals to humans.[4]

The genus *Acinetobacter* is Gram-negative coccobacilli belongs to the *Moraxellaceae* family, which is comprised of more than 59 classified species. These common sources of isolation are; environment, animals, and

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humans. *A. baumannii* is the leading cause of human infections like; pneumonia, meningitis, bacteraemia, endocarditis, skin and wound infections and urinary tract infection.[5-7] However, it can be traced out from a range of food items, like; raw vegetables, meat, fruits, milk, and milk products.[8,9] Most of the investigations have targeted the pathogenicity, molecular epidemiology, resistance mechanisms and fitness aspects of *A. baumannii*.[10,11] In India, there are a very few reports of *A. baumannii* in animals and meat samples and only a limited number of studies have reported such cases.[12-14] Several food-borne diseases outbreaks have resulted due to intake of raw or partially cooked meat.[15,16] Community associated infections[17] of *A. baumannii* are quite rare as per recent investigations, which in turn has got varied prevalence depending upon different continents and the detection methods utilized; from 10.4% in the United States, 4% in Hong Kong, and 0.5% to 3% in Europe.[18-20]

Acinetobacter species are ubiquitous, saprophytic bacteria, which can found on various environmental sources including water, soil and crop fields. However, *A. baumannii* has been specifically traced in healthcare settings more prevalently and has emerged as a nosocomial pathogen of priority concern due to its ability for thriving on a wide range of environment like; moist and dry hospital surfaces.[6,21] In the due course of research on *A. baumannii,* there are seldom reports of isolation the latter from milk, dairy products, food products and vegetables and meat.[16,6,22]

Although, a huge data is available on *A. baumannii* epidemiology in healthcare associated fatalities but environmental associated studies are lacking on it, which demands the futuristic investigations on environmental screening of the latter.[23,11] Association Studies of *P. aeruginosa, A. baumannii*, *E. coli, E. cloacae* isolates with food-borne diseases are quite inadequate. Therefore, we investigated herein the isolation, phenotypic characterization and prevalence of meat contaminating bacteria targeting few opportunistic pathogens like; *A. baumannii*, *E. coli, P. aeruginosa* among others from retail shops of North India.

MATERIALS AND METHODS

Sample Collection and Processing

A total of 30 meat sample from retail chicken meat shops, surface samples and knife samples were collected for isolation in the Mullana territory from January to March 2021. Approximately 1 g of meat was weighed and passed into in 9 ml of nutrient broth followed by homogenization for 1 min and incubated at 37°C for overnight in the incubator shaker. 10 µl of overnight grown bacterial culture was further inoculated on selective agar, which supported *A. baumannii* growth followed by incubation at 37°C overnight as described previously.[6] All the presumptive colonies were transferred onto MacConkey agar plates followed by incubation at 37°C for overnight. Different biochemical assays were performed to identify various bacterial species. Additionally, Gram staining was used to identify bacterial colonies along with analysis of colony morphology. Species level identification was done by MALDI-TOF MS using the standard protocol (Bruker Daltonics, Germany).[24,25]

CFU Count

1 ml of each of the samples (as prepared above) were mixed with 9 ml normal saline and homogenized well to get a uniform mixture. Serial dilution was performed for each sample from 10^{-1} to 10^{-6}. 100µl of each of the six dilutions of each sample were poured onto the MacConkey media plates and spreaded with the help of glass L-shaped spreader. Inoculated plates were incubated at 37°C for overnight. Next day, number of colonies was counted for each samples and dilutions. CFUs were counted for each sample by multiplying the number of colonies with dilution factors. For surface and knife swab samples, each swab was immersed initially in 10 ml of normal saline and followed by serial dilutions and spread plate culture. CFUs were counted as discussed previously.

MALDI-TOF MS

Bacteria isolates were cultured on Maconckey agar plates for 24 hr at 37°C. After overnight growth, isolated colonies of the bacteria were used as target sample for MALDI-TOF. All the bacterial samples were processed as per the manufacturer's instructions. The Microflex LT MALDI-TOF MS (Bruker Daltonics, Germany) instrument was used for identification process. In the sample preparation process, isolated bacterial colony of freshly grown culture was picked with the tip of sterile wooden toothpick and a uniform smear was prepared onto a steel biotarget plate in duplicates as described earlier (Direct Transfer procedure).[25] Let the sample be air dried for around 10 min. After air drying, 1.0 µl of matrix solution of –cyano-4-hydroxycinnamic acid (HCCA) was transferred over the dried uniform bacterial film. 1.0 µl of formic acid (100%) was transferred on another replicate for on-plate extraction method.[26] Again, let the sample be air dried at room temperature before inserting the analysing plate into the MALDI

machine. MALDI Machine automatically analyse the samples with laser shots and spectra were generated and received and compared with reference libraries to match the identity of the target strain. MALDI Biotyper database version 3 (Bruker Daltonics, Germany) was used to analyse the generated spectra and interpretation of cut-off values.[24, 27,25] 1.7000 to 1.999 cut-off value correctly determine the genus level and a value of ≥2.0 for species level of the organism.

Antibiotics Disk Diffusion Assay

The antibiotics susceptibility patterns of these bacterial isolates against some traditional antibiotics were examined by measuring the zone of inhibition using Kirby Bauer disk diffusion assay. Mueller Hinton agar plates were used for growing lawn culture of test bacterial isolates. After incubation overnight, zones of inhibition were measured for each antibiotic of each isolate. Netillmicin, Cotrimoxazole, Tetracycline, Erythromycin, Amikacin, Gentamicin, and Tobramycin were tested for each bacterial isolates. CLSI guidelines were followed for interpretation of the zone diameter and susceptibility pattern.[28]

RESULTS

A total of 13 isolates were collected from 50 meat samples, knife swabs and surface swabs of chicken slaughterhouses. Out of 13, 5 isolates were *E. coli*, 4 were *A. baumannii*, 2 were *E. cloacae*, one was *P. aeruginosa* and one was *P. stuartii* (Table 1). All the five types of bacterial isolates showed different colony morphology on MacConkey agar media plates. All the isolates showed variable susceptibility for 7 different antimicrobials (Table 1). MALDI-TOF MS correctly identified the bacteria up to species level (Table 1). All were identified with a good score. CFU count reveals that a heavy burden of bacterial load was present in the all the meat, knife and surface samples of the territory (Table 2). Heavy bacterial load was reported for *E. coli* followed by *A. baumannii*. All the isolates were tested susceptible for all the tested antimicrobials except erythromycin. One strain of *E. cloacae* was resistant to all the tested antibiotics. Although we collected the sample from a limited geographical location that too in a single season, we were able to isolate 4 most common pathogenic bacteria, which indicate the alarming situation of probability of an outbreak. Therefore, much needed

Table 1: MALDI Results and Antimicrobial Susceptibility Testing using Disk Diffusion Assay of bacterial isolates of Meat.

S. No.	Isolate ID	MALDI Results	Source	MALDI Score Value	Antimicrobial Susceptibility Testing (Disk Diffusion Assay)						
					Netillmicin	Cotrimoxazole	Tetracycline	Erythromycin	Amikacin	Gentamicin	Tobramycin
1	M01	*Enterobacter cloacae*	Meat	2.15	R	R	R	R	R	R	R
2	M02	*Acinet obacte r baumannii*	Surface	2.161	S	S	R	S	S	S	S
3	M05	*Acinet obacte r baumannii*	Meat	2.02	S	S	S	S	S	S	S
4	M08	*E. coli*	Knife	2.074	S	S	R	R	S	S	S
5	M09	*E. coli*	Knife	2.271	S	S	R	R	S	S	S
6	M10	*Enterobacter cloacae*	Knife	2.078	S	S	R	R	S	S	S
7	M11	*E. coli*	Surface	2.22	S	S	S	R	S	S	S
8	M12	*Acinet obacte r baumannii*	Meat	2.33	S	S	S	S	S	S	S
9	M13	*Acinet obacte r baumannii*	Knife	2.31	S	S	S	R	S	S	S
10	M15	*Provid encia stuartii*	Surface	1.91	S	S	S	R	S	S	S
11	M16	*E. coli*	Knife	2.38	S	S	R	S	S	S	S
12	M17	*Pseudomonas aeruginosa*	Meat	2.26	S	R	R	R	S	S	S
13	M18	*E. coli*	Meat	2.01	S	S	S	R	S	S	S

Table 2: Average CFU counts of different bacterial isolates of meat samples.

S. No.	Isolate ID	Bacteria	CFU Count / ml
1	M01	*Enterobacter cloacae*	2.95×10^3
2	M02	*Acinet obacte r baumannii*	5.50×10^2
3	M05	*Acinet obacte r baumannii*	6.89×10^2
4	M08	*E. coli*	2.35×10^4
5	M09	*E. coli*	2.56×10^4
6	M10	*Enterobacter cloacae*	1.94×10^3
7	M11	*E. coli*	3.15×10^3
8	M12	*Acinet obacte r baumannii*	1.69×10^3
9	M13	*Acinet obacte r baumannii*	2.34×10^3
10	M15	*Provid encia stuartii*	1.39×10^2
11	M16	*E. coli*	2.64×10^4
12	M17	*Pseudomonas aeruginosa*	1.85×10^3
13	M18	*E. coli*	2.95×10^4

surveillance in the area under study is required. A total of 5 samples were retrieved from meat samples, 5 from the knife swabs and 3 from surface swab samples. *A. baumannii* was isolated from all the three specimens. *E. cloacae* was isolated from meat and knife samples only. Except one sample from meat, all the 4 *E. coli* were isolated from surface and knife samples. Single *Providencia stuartii* strain was isolated from surface swab specimen.

DISCUSSION

In the present study a total of 12 bacterial isolates found belonging to *Enterobacteriaceae* family, which suggested the high prevalence of coliforms in the study area meat samples. To the best of our knowledge, this is the first study of its kind from India where *A. baumannii* is isolated from Meat sample obtained from retail meat shops. *A. baumannii* is the leading cause of human infections like; pneumonia, meningitis, bacteraemia, endocarditis, skin and wound infections and urinary tract infections.[6,7,29,30] However, it can be traced out by Askari *et al.* from a range of food items, like; raw vegetables, meat, fruits, milk, and milk products in Iran.[8,9] *A. baumannii* strains have been recently isolated and characterised from different meat samples in Iran by Askari *et al.* which showed the prevalence of high prevalence of antimicrobial resistance like healthcare settings.[31] In India, several investigations have previously detected *A. baumannii* in different nosocomial infections but no report is there from the other sources especially from chicken meat. As per PubMed search, there are ample reports of prevalence of *A. baumannii* from meat samples, In only one report from India, Singh *et al.* has highlighted the prevalence of *A. baumannii* from goat meat sample.[13] Highest prevalence of resistance against gentamicin (87.17%), were reported in this study followed by tetracycline (79%), erythromycin (74%), azithromycin (67), ciprofloxacin (59%), trimethoprim/sulphamethoxazole (56%) and rifampin (51%). However, in our investigation, only 1 isolate if *A. baumannii* was reported resistant to tetracycline only and another one to erythromycin.

In a study from Switzerland, *Lupo et al.* detected 25% isolates of *A. baumannii* from a total of 248 commercial raw meat samples, which belonged to genetically diverse clonal complexes with 29 different sequence types (STs).[32] However, relatively low antimicrobial resistance was reported in this study. In 2019, *Mari-Almirall et al.* reported 12 isolates of pathogenic *Acinetobacter* species including one *A. baumannii* and one novel spp. from 2 meat markets in Peru, which were highly susceptible to different antimicrobials.[33] *A. dijkshoorniae* was isolated in the same study for the first time from meat sample. Actual burden of *A. baumannii* in our study is quite higher if we compare the data with the other published studies. The prevalence of *A. baumannii* is escalating all over the globe, which often leads to hospital infections outbreaks.[34,35] However, the function of raw meat as a reservoir for *A. baumannii* remains indistinguishable. More studies are required to draw a clear graph of actual burden of *A. baumannii* in the meat samples.

E. coli is predominantly a commensal of humans, mammals and birds, where it is considered as an opportunistic pathogen. It is found in various habitats including sediments and water, poultry etc. Toxin producing *E. coli* are important food-borne pathogens worldwide. Most of the studies have highlighted the prevalence of *E. coli* in clinical settings but a few have investigated the environmental samples as well.[36] In the present study, *E. coli* has also been isolated in 5 of 13 samples of chicken meat, which underpins the growing public health concern. In the last 10 years, a total of 16 reports have highlighted the prevalence of *E. coli* in chicken meat in India as per Pubmed Search. In a study from South India, *Thangavel et al.* reported the isolation of several microorganisms including *E. coli* from refrigerated (stored) chicken meat.[37]

E. cloacae is extensively found in nature (water, food, soil, and sewage), but it is also observed in the human clinical specimens. It also causes various infections like; endocarditis, bacteremia, septic arthritis, UTIs, osteomyelitis, and respiratory tract infections in

healthcare sector.[38] This Gram-negative bacteria has been mostly associated with outbreaks of infections in Europe. Such hospital outbreaks are often occurred due to colonization of certain operative cleaning solutions and surgical equipment. As per Pubmed search, a total of 16 studies have previously investigated the *E. cloacae* from chicken meat all over the globe with not a single report from India. In the present study, 2 of 13 isolates of *E. cloacae* are identified in a total of 50 samples.

P. aeruginosa is yet another isolate, which was isolated in one the samples. The broad prevalence of *P. aeruginosa* infections has witnessed huge burden over public health in last couple of decades because of the associated deadly infections (urinary tract infections, bacteremia, and pneumonia). We have previous experience of isolation of one of the *Pseudomonas* spp. clinical specimens, which was once thought to be isolated solely from the environment.[39] Isolation of *P. aeruginosa* from meat sample suggested the probable transmission of this pathogenic strain from clinical to environmental settings. Till date, only 27 studies have investigated the *P. aeruginosa* from chicken meat samples throughout the world (source: Pubmed) with no reports from India.

CONCLUSION

The present study was aimed to isolate different bacterial isolates from raw chicken meat in the Mullana territory of North India. We observed *E. coli, A. baumannii, E. cloacae and P. aeruginosa* in different meat samples. Isolation of such pathogens from meat samples suggested the probability of their transmission from clinical settings to other environment. Significant resistance was observed against tetracycline and erythromycin antimicrobials. Because of the high prevalence of pathogens in the meat samples in the region, dedicated regular meat quality monitoring are recommended. Our study suggested the more studies with large number of samples are suggested for further assessment of bacterial isolates of meat in the same geographical area, which will certainly determine the actual burden of bacterial load. Safe handling of food and proper cooking are recommended to decrease or eliminate the risk of food poisoning due to meat.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ABBREVIATIONS

MDR: Multi-drug resistance; **ESKAPE:** Enterococcus species, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter species; **MADLI-TOF-MS:** Matrix assisted desorption ionization time of flight mass spectrometry; **CFU:** Colony forming unit; **HCCA:** Cyano-4-hydroxycinnamic acid.

SUMMARY

- The aim of present study was to isolate the bacterial pathogens from chicken meat samples.
- Numerous bacteria were isolated from meat, surface and knife samples with the help of MALDI-TOF MS.
- The dominant pathogenic bacterial isolates were *E. coli* and *A. baumannii*. Our study identified numerous fecal pathogens in the meat samples from rural area of North India, which underpins the dire need of tracing the source of potential contamination.

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