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RESEARCH ARTICLE

Occurrence and Antimicrobial Susceptibility of *Proteus mirabilis* from Chicken Carcass

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A B S T R A C T

The present study aimed to evaluate the prevalence of *Proteus mirabilis* (*P. mirabilis*) in poultry meat and to find its antimicrobial susceptibility. *P. mirabilis* has been frequently isolated from poultry and poses potential threat to public health. The pathogen resides in broiler's intestine, so it can be a source of contamination of chicken carcass in a slaughterhouse. A total of 50 samples of chicken carcass (from liver n=15, from intestine n=15, from thigh n=11 and from wings n=9) were taken from Faisalabad and cultured on Xylose Lysine Deoxycholate agar (XLD) for isolation and purification of *P. mirabilis*. Percent positivity of *P. mirabilis* in liver was found to be 60%, 46% in intestine, 36% in thigh and 33% in wings. *P. mirabilis* isolates showed high resistance to tetracycline (100%) and piperacillin (91.3%) while low resistance was shown to ceftazidime (8.6%). The study concluded that chicken could be the source of *P. mirabilis* and it can be a source of infection in human and animals.

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INTRODUCTION

Poultry remains the world largest domestic animal stock in terms of animal number (Ghonaim *et al.,* 2020). In Pakistan, the industry has expanded extensively in commercial levels as well as in household traditional levels (Memon *et al.,* 2021). In all meat producing countries of the world, Pakistan ranked 11th where poultry contributed almost 35% of total meat production. More over three million peoples are working directly in poultry industry, in order to meet the major protein sources for whole population of the country which offers the largest supply of eggs and meat. Infectious diseases have always posed an important health and economical risk to poultry industry of Pakistan (Abadeen *et al.,* 2021).

P. mirabilis is ubiquitous, Gram negative rod found in poultry, soil, sewage, water, fecal matter and is considered as the normal micro flora of gastro intestinal tract (GIT) of humans and animals (Sanches *et al.,* 2020). Proteus species are the common cause of Urinary tract infections (UTIs) and are among the most common bacterial infections acquired from hospital and community (Bilal *et al.,* 2019). According to CDC report 75% of them are catheter associated urinary tract infections (CAUTIs) (CDC 2017). CAUTIs are a predominant nosocomial infection in the US and are especially common in long-term catheterized patients. Epidemiological data illustrates that *P. mirabilis* is second common cause of catheter-associated bacteremia (15%) and third common cause of UTI's (12%). Among Proteus infections, 90% of infections are caused by *P. mirabilis* and are mostly seen in immunocompromised people (Yuan *et al.,* 2021). In Pakistan, prevalence of this bacterium is 13.8% in UTI's (Ullah *et al.,* 2018).

P. mirabilis is considered to occur in various food products; in meat items such as chicken and pork, its occurrence in environment and food can lead to human clinical infections (Ram *et al.,* 2019).

Isolation of Proteus spp. from poultry droppings along with other enteric bacteria facilitates the transmission of bacteria from feces to the slaughter line and cross-contamination in evisceration process. Cross-contamination may permit the transmission of antimicrobial- resistant bacteria from food to humans (Lim *et al.,* 2021).

Contamination of poultry and poultry products with pathogens present in fecal matter such as Proteus, Salmonella, Klebsiella, Pseudomonas, Enterobacter and *E. coli* exhibit problems in food processing and hygiene that also leads to spoilage of poultry meat and spread of pathogens to consumers (Danbappa *et al.,* 2018). Commonly, diseases caused by *P. mirabilis* are treated with extended-spectrum cephalosporins while fluoroquinolones and aminoglycosides are also used (Iraqi *et al.,* 2021).

Emergence of highly resistant strain of *P. mirabilis* can menace public health. Globally several studies have evaluated MDR *P. mirabilis* from poultry meat; but limited studies are present in Pakistan for poultry samples. The current study was designed to check the occurrence of *P. mirabilis* in different organs of poultry and to evaluate antimicrobial resistance of *P. mirabilis* isolated from chicken carcass.

MATERIALS AND METHODS

Sample collection from chicken carcasses: A total of 50 samples of chicken (5-6 weeks old) carcasses (n=15 liver, n=15 Intestine, n=11 thigh and n=9 wings) were taken aseptically from diagnostic pathologic laboratory of UAF and retail markets of Faisalabad, Pakistan. The samples were collected in a sterile bag having buffered peptone water. After collection samples were transported immediately to Institute of Microbiology at 4°C for further processing.

Processing of samples: For the isolation of *P. mirabilis* from food samples still there is no standard method so we use Salmonella isolation protocol according to Food and drug administration manual (Ho *et al.,* 2013). Briefly, 25g of chicken carcass was placed in stomacher bag having 100ml buffered peptone water and homogenized for 5 min. Homogenate was then incubated for 24 hours. Loopful of specimen was streaked on pre-poured plate of Xylose lysine deoxycholate agar and incubated for 24hrs at 37°C. To observe swarming growth, colonies were re-cultured on Blood agar.

Phenotypic examination of *P. mirabilis* from chicken carcasses: Bacterial colonies were characterized on the basis of morphology and colony characteristics on XLD agar. Microscopic characterization was done by performing Gram's staining. *P. mirabilis* was differentiated from P. vulgaris by Indole negative test. For further confirmation Biochemical tests (urease test, catalase test, motility test, Methyl red and oxidase test) were performed.

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing was performed on *P. mirabilis* isolates by Kirby Bauer disc diffusion method. According to the Clinical and Laboratory Standards Institute (CLSI, 2019) guidelines the isolates of *P. mirabilis* were classified as resistant, intermediate and susceptible on the basis of zone of inhibition. Antibiotic discs (Oxoid, UK) including tetracycline 30µg, Ceftazidime 30µg, Sulfamethoxazole 25µg, Piperacillin 100µg and Ciprofloxacin 5µg were applied. Occurrence and percentage positivity of *P. mirabilis* was calculated from studied isolates and antibiotic sensitivity was presented in percentage.

RESULTS

Isolation and confirmation of *P. mirabilis*: Out of 50 samples of chicken carcasses 23 were confirmed for microbial growth of *P. mirabilis* and 27 samples showed no growth. Phenotypic characteristics of *P. mirabilis* on XLD agar showed pink color colonies with black center as shown in Fig. 1. On blood agar *P. mirabilis* showed specific swarming growth with fishy smell as shown in Fig. 1. All isolates of *P. mirabilis* (n=23) were positive for urease, methyl red, catalase and motility test. *P. mirabilis* was differentiated from P. vulgaris by indole negative test. All isolates of *P. mirabilis* (n=23) were negative for indole and oxidase test.

Percentage positivity of *P. mirabilis* in organs of chicken carcasses: Out of 50 samples *P. mirabilis* weas isolated from 23 samples. Out of total samples of liver (n=15) 9 samples were positive for *P. mirabilis*, out of 15 samples of intestine 7 samples were positive, out of 11 samples of thigh 4 samples were positive and out of 9 samples of wings 3 were positive. The percentage positivity of *P. mirabilis* in liver, intestine, thigh and wings was 60, 46, 36 and 33% respectively as shown in Table 1.

Antimicrobial susceptibility pattern of *P. mirabilis* isolated from chicken carcasses: All positive isolates of *P. mirabilis* (n=23) showed 100% resistance to tetracycline, resistance to piperacillin was 91%, resistance to ciprofloxacin was 82%, resistance to sulfamethoxazole 43% and resistance to ceftazidime was 8%. High sensitivity was shown by ceftizidime 86% followed by sulfamethoxazole (26%) as shown in Fig. 2. The *P. mirabilis* isolates were intermediately susceptible to sulfamethoxazole (30%), ciprofloxacin (13%) and piperacillin (8%).

DISCUSSION

Enteric pathogens are responsible of causing diseases that affect broiler and leads to severe economic losses in poultry sector (Mehmood *et al.,* 2020). *P. mirabilis* is frequently present in the intestinal tract of animals and humans. Animal is a major source for the transmission of this pathogen to human because contamination of chicken carcass with intestinal flora is very common when the carcasses is placed in chiller for washing and cooling in slaughterhouse (Sanches *et al.,* 2021).

In Pakistan information regarding MDR Gram Negative *P. mirabilis* in chicken carcass is rarely available. The present study revealed the occurrence and antimicrobial sensitivity of *P. mirabilis* in chicken carcass.

The present study was performed by taking 50 samples of chicken carcass (15 livers, 15 intestines, 11 thighs and 9 wings) from different areas of Faisalabad, Pakistan. Similar study was performed to determine the prevalence of *P. mirabilis* in chicken carcass and its susceptibility to major antibiotics by taking 707 samples

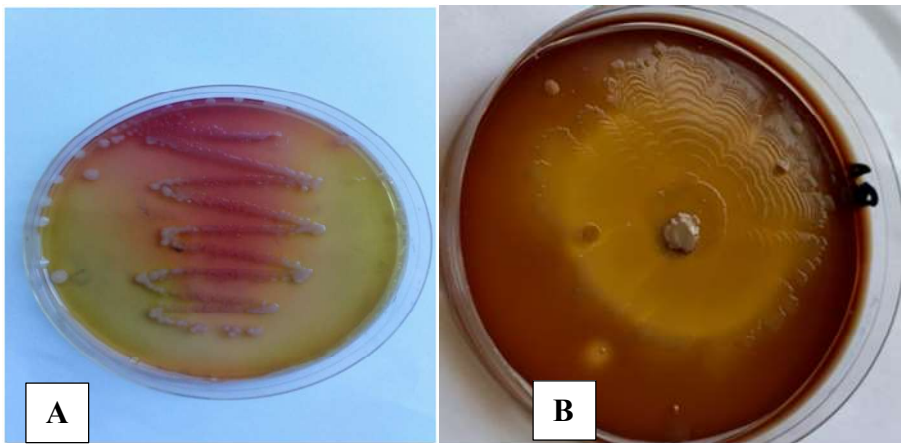

Fig. 1: Representative agar colony morphology of *P. mirabilis* isolated from chicken carcasses on specific media A) XLD agar and B) Blood agar.

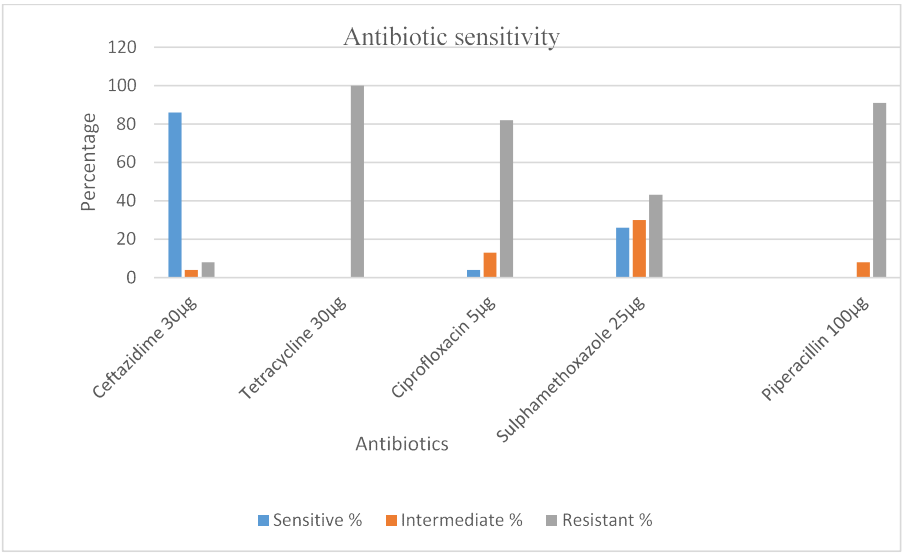

Fig. 2: Graphical representation of antibiotic sensitivity of *P. mirabilis* isolated from chicken carcasses.

Table 1: Percentage of *P. mirabilis* in different poultry organs of chicken carcasses

Organ	No. of sample examined	Number of confirmed isolates of *P. mirabilis*	% age poultry
Liver	15	9	60
Intestine	15	7	46
Thigh	11	4	36
Wings	9	3	33

of fresh raw chicken carcasses (Li *et al.,* 2022). The percentage positivity differences may be attributed to meat processing practices and hygienic conditions of countries.

Present study showed that percentage positivity of *P. mirabilis* in chicken carcass was 46% (23/50) which is justified by study conducted in Lebanon. Another study also exposed the percentage positivity of *P. mirabilis* 36.25% which was similar to our findings (Yu *et al.,* 2021).

Our study showed that high prevalence rate of *P. mirabilis* was found in liver (60%). Present study showed that distribution of *P. mirabilis* in small intestine was 46% (7/15). Similar study was performed in which distribution of this bacterium in small intestine of clinically sick birds was 25%. Moreover, study also revealed that *P. mirabilis* generally spread in the visceral organ of clinically sick birds (Ajayi *et al.,* 2018).

In our study *P. mirabilis* showed highest resistance to tetracycline (100%), sulfamethoxazole (91%) and lowest resistance to ceftazidime. Highest sensitivity was shown by isolates to ceftazidime (70%) and lowest sensitivity to sulfamethoxazole (4%). A study showed the susceptibility of *P. mirabilis* to cefotaxime, cefoxitin and cefepime. However, resistance to sulfamethoxazole was 69% (Han *et al.,* 2020). Another study was performed in India to check antimicrobial susceptibility pattern of *P. mirabilis*, results of the study showed that isolates were highly resistant to cefotaxime, ampicillin, gentamicin and sulfamethoxazole (Matheus *et al.,* 2019).

However, irrational use of antibiotics is big menace which results in the emergence and dissemination of MDR strains of non-pathogenic and pathogenic organisms that may be transmitted to humans by food chain. The prompt surge in the development and transmission of resistance is the major cause of concern. Thus, for efficient treatment and control measures testing of isolated pathogens for antimicrobial resistance has become a global interest.

The current study concluded that poultry meat sold in traditional markets has already been contaminated with *P. mirabilis* and high level of antibiotic resistance of this pathogen may pose a serious threat to public health. Therefore, more surveillance and studies will be needed in Pakistan to monitor the antibiotic resistance of *P. mirabilis* in food and new strategies should be devised to

stop the progress of antibiotic resistance in foodborne *P. mirabilis*.

Authors contribution: KI, MIA: Conceived the idea, experimentation and study design, write the paper; AA, RA, SA, AR: Proof-read and correct the paper, data analysis; MAA, MAS, BA, NS: Data analysis, study design and proof-reading.

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