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SHORT COMMUNICATION

# Isolation of multi-drug resistant *Klebsiella* sp. from bovine mastitis samples in Rangpur, Bangladesh

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## ABSTRACT

**Objective:** The objective of this study was to identify the multi-drug resistance (MDR) *Klebsiella* sp. from mastitis milk samples.
**Materials and Methods:** In the current research, 48 clinical mastitis milk samples were collected from Rangpur division, Bangladesh. Confirmation of bovine mastitis (BM) was done by the California Mastitis Test (CMT). All the CMT positive isolates were subjected for the identification of *Klebsiella* sp. using through a series of cultural and biochemical tests. MDR *Klebsiella* sp. isolates were determined using the disk diffusion method, and minimum inhibitory zones were measured by following Clinical and Laboratory Standards Institute. MDR patterns of the isolates were also subjected to study by using housefly (*Musca domestica*).
**Results:** Among the isolates, 62.5% (*n* = 30/48) revealed the presence of *Klebsiella* sp. Eight antimicrobial agents including Amoxicillin, Novobiocin, Erythromycin, Vancomycin, Cephradine, Tetracycline, Bacitracin, Methicillin, and housefly (*M. domestica*) showed complete resistance to *Klebsiella* sp. On the other hand, Chloramphenicol, Gentamicin, Ciprofloxacin, Azithromycin, Norfloxacin, Levofloxacin, and Nalidixic acid showed sensitivity.
**Conclusion:** This study helps to treat BM with effective antibiotics and helps in an epidemiological study in Rangpur division as well as helps to create public health awareness.

## ARTICLE HISTORY

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## Introduction

Antimicrobial resistance is an ultimate threat to the animal as well as a human being throughout the world. Bovine mastitis (BM) is caused by a variety of bacteria; among them, *Klebsiella* sp. is an important Gram-negative pathogen which may initiate emerging incidence [1,2]. *Klebsiella* sp. is an opportunistic bacterium that can cause primary bacteremia as well as urinary tract infection in human and animal [3,4]. Fey et al. [5] reported that *Klebsiella* sp. has zoonotic importance. *Klebsiella* sp. is notoriously appeared in dairy food products [6], and it is reported that they are responsible for clinical as well as subclinical BM [7]. It is quite difficult to control BM originated from *Klebsiella* sp. infection [4]. As reported by Grohn et al. [8], milk production falls and mortality increased in cows affected with *Klebsiella* sp. They are able to produce a significant loss in the dairy farm by reducing production; which is considered as more fatal as compared to infection caused by *Escherichia coli* [9].

Extensive use of antibiotic leads to the development of multi-drug resistance (MDR) organisms. The rate of MDR organism development is increasing day by day [11]; the development of MDR *Klebsiella* sp. is also gradually increasing worldwide [11]. Consequently, both antibiotic treatment and mass vaccination showed limited effects against BM caused by *Klebsiella* sp. [12]. Increasing MDR bacteria and their treatment with antimicrobial agents as well as zoonotic importance are considered as important issues globally [13,14]. Constrained examines have been completed on the detachment of *Klebsiella* sp. in Bangladesh [15,16]. Previously, we isolated and identified *Klebsiella* sp. by conventional bacteriological techniques.

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The present study focused on molecular detection of mastitis-causing *Klebsiella* sp. from clinical mastitis milk samples in Rangpur division, Bangladesh, and the antibiotic susceptibility patterns of the organism were investigated for the first time in Bangladesh.

## Materials and Methods

### *Collection and preparation of samples*

A total of 48 milk samples were gathered from the selected BM dairy cows in Rangpur division, Bangladesh. The samples were collected based on clinical sign and inflammatory lesion of udder and teat. About 10–15 ml sample was collected from each dairy cow. Immediately after collection, the California Mastitis Test (CMT) was done according to Schalm and Noorlander [17] for the confirmation of BM. All the suspected samples were aseptically transferred to the Microbiology Laboratory, Hajee Mohammad Danesh Science and Technology University (HSTU) by maintaining a cool chain for microbiological analysis.

### *Isolation and identification of Klebsiella sp.*

Samples were cultured on nutrient agar (NA), Eosin Methylene Blue (EMB) agar, and MacConkey (MC) agar at 37°C for 24 h. Isolation and identification were done by conventional techniques according to Edwards and Ewing [18]. Furthermore, the isolates were biochemically confirmed based on Merchant and Packer [19].

### *Antimicrobial susceptibility testing*

Disk diffusion method [20] was used to determine the MDR *Klebsiella* sp. from the isolates using MHA (Hi-Media, India), and the zone of inhibition was interpreted according to standards of the National Committee for Clinical Laboratory Standards [21]. A total of 15 antibacterial disks (Hi-Media, India) were used in this study, namely, Gentamicin (GEN 10 µg), Amoxicillin (AMX 30 µg), Chloramphenicol (C 30 µg), Ciprofloxacin (CIP 5 µg), Bacitracin (B 10 µg), Azithromycin (AZM 30 µg), Erythromycin (E 15 µg), Methicillin (Met 5 µg), Novobiocin (NV 30 µg), Vancomycin (VA 30 µg), Norfloxacin (NX 10 µg), Tetracycline (TE 30 µg), Levofloxacin (LE 5 µg), Nalidixic acid (NA 30 µg), and Cephradine (CH 30 µg). The zones were estimated in millimeter and resistance and susceptibility were recorded [22]. These MDR *Klebsiella* sp. were also studied by using housefly on MHA media and observed their antimicrobial activity. On the other hand, Nazari et al. [23] studied with housefly maggot extracts, but here we applied the whole fly.

## Results

The collected samples were inoculated on NA in which they produced large, circular, smooth, and convex colonies. Round, pink, slightly raised, translucent, and mucoid colonies were found in MC, and on EMB they also showed mucoid pink colonies. Then Gram-negative, short rod with capsule *Klebsiella* sp. was observed under a microscope. The identified isolates were subjected to a biochemical test for more confirmation (Fig. 1). In methyl-red test and indole test, the isolates were produced a negative result. The Voges–Proskauer test, Simmon's citrate test, and catalase tests were positive for *Klebsiella* sp. On Triple Sugar Iron (TSI) test, the slant was yellowish with no changes in butt and no H2S produced, but gas bubble appeared.

Antimicrobial susceptibility test of *Klebsiella* sp. (Fig. 2) reveals that this organism was MDR of which AMX, B, E, MET, NV, VA, TE, and CH were completely resistant. Out of 15 antibiotic agents, GEN (19 mm), C (24 mm), CIP (30 mm), AZM (25 mm), NX (25 mm), LE (22 mm), and NA (17 mm) were showed above-mentioned zone of inhibition in mm. The positive *Klebsiella* sp. was studied using housefly and showed no zone of inhibition.

## Discussion

In the present research work, 62.5% ($n$ = 30) BM involved with *Klebsiella* sp. could be detected based on CMT, cultural, and biochemical tests. After the collection of mastitis milk, samples were transferred to the laboratory maintaining the cool chain. Then, grown into NA, EMB, and MC, respectively, by following Edwards and Ewing [18]. From the cultural and Gram staining test, Gram-negative, rod-shaped, and non-motile *Klebsiella* sp. were identified. From the pure culture, several different biochemical tests were performed for the confirmation of *Klebsiella* sp.

*Klebsiella* sp. was notoriously and ubiquitously appeared in milk along with their products that have zoonotic importance [14]. In this research work, 30 samples were positive for *Klebsiella* sp. The prevalence of *Klebsiella* sp. in the current study was higher than the study of Gundogan and Yakar [24] and Haryani et al. [25]. This variation might be due to geographical distribution, biosecurity, and immunological status of the study population.

Antibiogram study revealed that all the isolates were showed MDR in which AMX, B, E, MET, NV, VA, TE, and CH were completely resistant to *Klebsiella* sp. which is supported by Gundogan et al. [11]. Then again, CIP (30 mm) produced the highest zone of inhibition and AZM, NX, LE, and NA were produced 25, 25, 22, and 17 mm zone of inhibition, respectively. In the present study, houseflies (*Musca domestica*) were caught and stored into PBS (Phosphate Buffer Saline) then directly placed on MHA (Mueller Hinton Agar) plates which were pre-stained with pure field isolates. Nazari et al. [23] worked with visceral parts of housefly maggot and its extracted material which showed good antimicrobial activity against different antibiotic agents. But, houseflies in the current research showed complete resistance during antibiogram study. This might be due

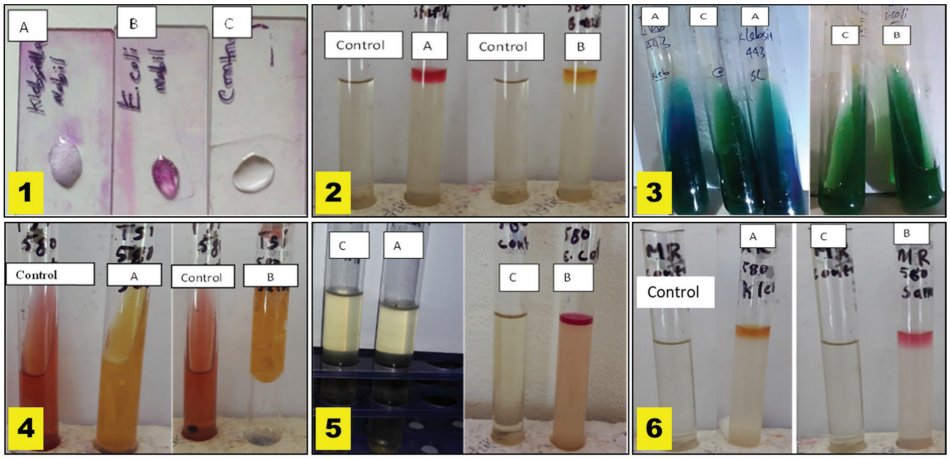


**Figure 1.** Biochemical tests for the identification of *Klebsiella* sp. (1) Catalase test; (A) *Klebsiella* sp., (B) *E. coli,* (C) control, (2) Voges–Proskauer test; (A) *Klebsiella* sp. positive, (B) *E. coli* negative (3) Simmon's citrate test; (A) *Klebsiella* sp. positive, (B) *E. coli* negative, (C) control (4) TSI test; (A) *Klebsiella* sp., (B) *E. coli*, (5) Indole test; (A) *Klebsiella* sp., (B) *E. coli*, (C) control, (6) Methyl-Red test; (A) *Klebsiella* sp. negative, (B) *E. coli* positive, (C) control.

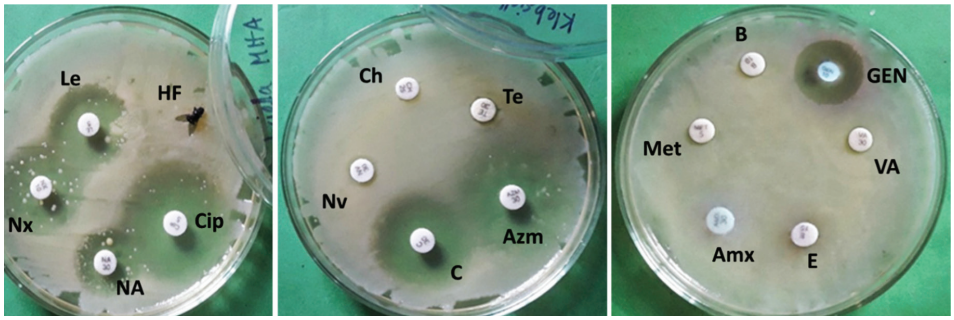


**Figure 2.** Antibiogram of *Klebsiella* sp. on MHA. Le = Levofloxacin, HF = Housefly, Nx = Norfloxacin, NA = Nalidixic acid, Cip = Ciprofloxacin, Ch = Cephradine, Te = Tetracycline, NV = Novobiocin, C = Chloramphenicol, Azm = Azithromycin, B = Bacitracin, Met = Methiclillin, Gen = Gentamicin, VA = Vancomycin, E = Erythromycin, Amx = Amoxicillin.

to the use of whole housefly and in external body parts of housefly carry several different organisms [26] which may be MDR. The deliberate use of antibiotic for the treatment of BM causes MDR which is a global issue. From this study, it is concluded that CIP, AZM, NX, LE, and NA can be the choice of drugs for treating the BM in Rangpur, Bangladesh.

## Conclusion

The prevalence of the *Klebsiella* sp. in mastitis milk was found as 62.5%. Current research work may help to choose a specific drug to treat BM and also helps to control the indiscriminate use of antibiotics that causes MDR.

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## Conflict of interest

The authors declare that they have no conflict of interests.

## Authors' contributions

MS, MRA, and MKH designed and interpreted experiments. MS conducted the actual experiments and prepared the draft of the manuscript. All the authors finally approved the manuscript for publication.

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