Molecular characterization of antibiotic resistant bacteria and assessment of antimicrobial residues in the dairy milk in India

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Introduction

• Food safety is a great concern in low- and middle-income countries. India is the largest milk producer in the world and milk is consumed by the most people.
• Antimicrobial resistance (AMR) is a serious threat to the health of both humans and animals, and resistant bacteria can also be spread through food.
• Therefore, the risk of exposure to antibiotic resistant bacteria and antibiotic residues in milk needs serious attention.
• This study was designed to investigate the presence of AMR bacteria and antibiotic residues in milk in two states in India, Assam and Haryana.

Materials & Methods

• Milk samples were collected from two Indian states during 2016-18.
• 729 samples (283 from Assam and 444 from Haryana) were analyzed for microbiological studies.
• 730 milk samples (330 from Assam and 400 from Haryana) were analyzed for antibiotic residues.

Fig 1: Map depicting the sampling states from India

• Bacteria were isolated as per standard protocol and subjected to antibiotic disc diffusion testing.
• Gram-positive bacteria were tested against cefoxitin (30µg), methicillin (5µg) and oxacillin (1µg) for screening methicillin resistance.
• Gram-negative bacteria were tested against cefoxitin (30µg), cefotetan (30µg), cefotaxime (30µg), ceftazidime (30µg), imipenem (10µg) and meropenem (10µg) was used for screening beta-lactamases (extended spectrum beta-lactamase, metallo-beta-lactamase, AmpC betalactamase).
• The isolates resistant by disc diffusion test were screened for methicillin resistance genes and beta-lactamases (ESBL, MBL, AmpC) genes using polymerase chain reaction.
• The collected samples were also analyzed for antibiotic residue testing using spore-based assay and Charm Rosa test.

Results

• In total, 256 resistant Gram-positive isolates were obtained by disc diffusion testing, most were identified as genus Staphylococcus, and 7% (17/256) of phenotypically resistant staphylococci harbored methicillin resistance mecA / meC genes by genotyping.
• The 17 methicillin resistant staphylococci identified, carried mecA (15) and meC (2) genes.
• Of the resistant Gram-negative isolates (148) obtained by disc diffusion testing, by genotyping 29% (n=43/148) were beta-lactamase isolates among E.coli, Klebsiella spp., Shigella spp. and other gram-negative bacteria.
• ESBL - 16% (7/43), AmpC - 60% (26/43), MBL - 14% (6/43).
• Some bacteria also had combinations of resistance genes: MBL+AmpC - 7% (3) and ESBL+ AmpC - 2% (1).

The prevalence of antibiotic residues in milk of Haryana was 8% (32/400) and in Assam was 5% (16/330).
• The antibiotic residues detected were novobiocin 46% (22/48), macrolides 27% (13/48), sulfa drugs 10% (5/48), tetracycline 10% (5/48), and beta-lactam 6% (3/48).

Antibiotic residue prevalence in Assam and Haryana, and the proportional distribution of the detected antibiotics

• Antibiotic resistant bacteria and residues were found in milk samples from both the states, but more commonly in Haryana compared to Assam.

Conclusions

• The study found both the occurrence of 7% methicillin resistance genes among isolated Staphylococcus spp, as well as 29% ESBL, MBL and AmpC producers among the isolated E.coli, Klebsiella and Shigella spp.
• Also, antibiotic residues were found in 7% of the milk samples.
• Since the milk sampled were all at the point of sales, these resistant bacteria could point to risks for human health, and even if the milk was boiled before consumption, the antibiotic residues could contribute to the risks for humans.
• Hence there is need for intensified awareness creation and surveillance on the occurrence of antimicrobial resistance and antibiotic residues in animals and food chain of animal origin to device strategies for preventing their spread.

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