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Antibiograms and Multi-Antibiotic Resistance of *Staphylococcus* and M*icrococcus* Species Isolated from Chlorinated Drinking Water Supply of the Mahaweli River, Sri Lanka

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Abstract

Contamination of antibiotic-resistant bacteria (ARB) in drinking water sources poses a growing threat to public health. Monitoring antibiotic resistance patterns in water systems is crucial for understanding the spread of bacteria with multi-antibiotic resistance (MAR). The present study focused on identifying antibiograms and MAR of predominant bacterial isolates from chlorinated drinking water collected from outlets of 14 water treatment plants along the Mahaweli River between Kotmale and Victoria reservoirs. Water samples were initially cultured on nutrient agar and *Staphylococcus* spp. (SC. spp.) (n=101, 74.3%) and *Micrococcus* spp. (MC. spp.) (n=35, 25.7%) were then identified using Gram staining and biochemical analyses. Antibiotic resistance of these isolates was evaluated using the Kirby Bauer disc diffusion method with a panel of 10 antibiotics; cefuroxime (CXM-30), ceftazidime (CAZ-30), augmentin (AUG-30), cefoxitin (FOX-30), gentamycin (GEN-10), tetracycline (TE-30), ciprofloxacin (CIP-5), chloramphenicol (C-30), clindamycin (CD-2) and co-trimoxazole (SXT-25), as referring to CLSI-2021 standards. Antibiograms showed highest resistance to CAZ-30 (n=80, 79.2% for SC. spp. and n=24, 68.6% for MC. spp.), a third-generation cephalosporin, and subsequent resistance to other commonly used antibiotics; CD-2 (n=45, 44.6%, for SC. spp. and n=19, 54.3%, for MC. spp.), C-30 (n=34, 33.7% for SC. spp.) and CXM-30 (51.4%, n=18 for MC. spp.). Resistance to AUG-30 and SXT-25 was notably low, with only 3.0% and 5.7% of SC. spp. and MC. spp. isolates respectively exhibiting resistance to each antibiotic. Only three (3.0%) SC. spp. and one (2.8%) MC. spp. isolates were sensitive to all tested antibiotics. Having resistance to two or more antibiotic classes, 61.4% (n=62) of SC. spp. and 62.9% (n=22) MC. spp. isolates exhibited MAR. Calculated multi-antibiotic resistance indices (MARI) for both isolates ranged from 0 to 0.71, where 21.0% (n=13 out of 62) and 9.1% (n=2 out of 22) of MAR isolates of SC. spp. and MC. spp., respectively were greater than 0.5, indicating the possibility of resistance to >3 of seven classes of tested antibiotics. Contamination with ARB and MAR isolates may indicate that chlorination alone is not effective in eliminating ARB from drinking water. The detection of MAR SC. spp. and MC. spp. in chlorinated drinking water supply raises concerns about the potential spread of ARB via treated water systems, with implications for public health.

Keywords: *Antibiotic susceptibility test, Disc diffusion method, Multi-antibiotic Resistance Index, Water treatment plants*