

Lead tolerance capacity of clinical bacterial isolates and change in their antibiotic susceptibility pattern after exposure to a heavy metal

Abstract

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Introduction: Heavy metal pollutions of soil and wastewater are a significant environmental problem as they are not degraded or destroyed. Several metal resistance mechanisms have been identified which is responsible for alteration of normal cell physiology leading to development of drug resistance in microorganisms. Heavy metals used in industry and in household products are, along with antibiotics, creating a selective pressure in the environment that leads to the mutations in microorganisms. The present study was carried out to study the heavy metal lead tolerance by bacteria and change in antibiotic-sensitivity pattern after its exposure. **Materials and Methods:** 30 clinical isolates from various samples received in the Department of Microbiology, Government Medical College, Surat, were included in the study. To check the lead tolerance capacity, isolates were exposed to graded concentration of lead nitrate by plate dilution method, starting from 50 up to 1000 µg/ml strength. Antibiotic susceptibility was performed by the Kirby Bauer disc diffusion method. A change in antibiotic susceptibility pattern was studied before and after lead exposure. **Result:** 30 clinical isolates were included in the study, 25 Gram negative (83.3%) and 5 Gram positive (16.7%). MIC to lead was higher in *Acinetobacter spp.* and *Pseudomonas spp.* (600-1000 µg/ml) as compared to *E. coli*, *Klebsiella spp.*, *S. aureus* (50-150 µg/ml). Multiple antibiotic resistance indexes were changed significantly after lead exposure. **Conclusion:** Bacteria exposed to high levels of heavy metals in their environment have adapted to this stress by developing various resistance mechanism. Infection with antibiotic-resistant organisms create problem in treatment and management of patients. We should take efforts to prevent environmental pollution with such heavy metals and transmission of antibiotic-resistant microorganism from environment to health care set up.

Key words: Bacteria, drug resistance, heavy metal, lead tolerance, multiple antibiotic resistance

INTRODUCTION

Heavy metal pollutions of soil and wastewater are a significant environmental problem as they are not degraded or destroyed.^[1] Some heavy metals are essential which are required by the organisms as micro nutrients and are known as “trace elements”, while some heavy metals do not play any role in metabolism so they are required in low amount. In nature, there are about 50 heavy metals of special concern because of their toxicological effect to human beings and other living organisms. Many agricultural and industrial practices led to environmental pollution by heavy metals. Heavy metals influence the organism’s population as well as it affects the growth, morphology, biochemical activities and leading to a decrease in biomass and diversity. Heavy metals can damage the cell membranes, alter enzymes specificity, disrupt cellular functions and damage the structure of the DNA.^[2-4]

Heavy metals can be accumulated and transferred to higher organisms in food chain and lead to serious ecological and health problem.^[5,6] To combat with heavy metal pollution in the environment, bacteria have evolved several resistance mechanisms that lead to persist them in the environment or to grow.^[5] Several metal resistance mechanisms have been identified which include exclusion by permeability barrier, intra and extra cellular sequestration, active transport, efflux pumps, enzymatic detoxification and reduction in the sensitivity of the cellular targets to metal ions.^[4] There are many

known mechanisms by which resistant traits are retained and propagated in the presence of elevated chemical stressors like quaternary ammonium compounds and heavy metals, which locally influence resistance markers in exposed microbial populations.^[7]

As man further contaminates his own environment, he alters the milieu of those organisms for whom he is the host. Heavy metals used in industry and in household products are, along with antibiotics, creating a selective pressure in the environment that leads to the mutations in microorganisms that will allow them better survive and multiply. There is also evidence to indicate that there may be a correlation between the emergence of resistance to antibiotics and heavy metals.^[4,8] The present study was carried out to study the heavy metal lead tolerance by bacteria and change in antibiotic sensitivity pattern after its exposure.

MATERIALS AND METHODS

Sample collection

The present study was conducted during April-May 2013, in microbiology laboratory at New Civil Hospital, Surat, after having ethical permission from institutional ethical committee. Clinical samples like pus, swab, urine, CSF, blood culture and ET secretion received from various ICUs, wards, OPDs were included.

Isolation and identification of isolates

Samples were cultured on blood agar and MacConkey agar for the isolation of microorganism. The culture plates were incubated for 24 hours at 37°C and were observed for growth. Characterization and identification of isolates were done on the basis of morphology and biochemical reactions and also by automated system (Vitek 2).^[9]

Antibiotic susceptibility testing of the isolates

Antibiotic susceptibility was performed by the Kirby Bauer disc diffusion method on Muller hinton agar. The drugs tested for Gram-positive isolates were gentamycin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), ceftazidime (30 µg), amoxicillin/clavulanic acid (20-10 µg), tetracycline (30 µg), chloramphenicol (30 µg), co-trimoxazole (25 µg), ampicillin/sulbactam (10/10 µg), oxacillin (1 µg), penicillin (10 Units), erythromycin (15 µg), clindamycin (2 µg), linezolid (30 µg), vancomycin (30 µg) and teicoplanin (30 µg). In Gram-negative isolates Gentamycin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), ceftazidime (30 µg), amoxicillin/clavulanic acid (20-10 µg), Tetracycline (30 µg), chloramphenicol (30 µg), co-trimoxazole (25 µg), piperacillin/tazobactam (100/10 µg), ampicillin/sulbactam (10/10 µg), ceftazidime (20 µg), ceftazidime/clavulanic acid (30/10 µg), ceftriaxone (75 µg), cefepime (30 µg), imipenem/cilastin (10/10 µg), nalidixic acid (30 µg), cefixime (5 µg), polymyxin-B (300 Units), netilmicin (10 µg), norfloxacin (10 µg), levofloxacin (5), cefoperzone (75 µg), colistin (10 µg) and aztreonam (30 µg) were tested according to suspected isolates. All the culture media and antibiotic discs were procured from Hi-media Pvt. Ltd, Mumbai. Result were interpreted as sensitive, intermediate and resistance as per recent CLSI guidelines, 2013.^[10]

Exposure to heavy metal^[11]

Isolated bacterial strains were exposed to lead nitrate Pb(NO₃)₂ solution (10 mg/mL) for heavy metal exposure. MIC was determined by the plate dilution method against lead by gradually increasing its concentration in MacConkey agar and Blood agar for Gram-negative and Gram-positive isolates, respectively. The initial concentration used was 50 µg/ml with a gradual increase until the strains failed to give colonies on the plate, which was 1000 µg/ml. The lowest concentration that prevented bacterial growth was considered as MIC for that isolate. Isolated heavy metal-resistant isolates from the last plate were tested for antibiotic susceptibility test to the same antibiotics by Kirby Bauer disc diffusion method. Results for antibiotic susceptibility pattern before and after lead exposure were recorded and analyzed for the change in pattern.

RESULTS

Thirty clinical bacterial isolates were included in the present study, out of which 25 were Gram negative (83.3%) and 5 were Gram positive (16.7%). Among Gram-negative isolates *Klebsiella spp.* (9), *E. coli* (5), *Acinetobacter spp.* (3), *Pseudomonas spp.* (3), *Proteus spp.* (2), *Salmonella typhi* (1), *Enterobacter cloacae* (1) and *Citrobacter spp.* (1) were isolated. Among Gram positive all isolates were *Staphylococcus aureus* (5). The isolates were from different clinical samples like urine (14), swab (10), blood culture (3), drain, CSF and ET secretion (1 each).

When the organisms were exposed to lead, it was observed that *Acinetobacter spp.* and *Pseudomonas spp.* had higher MIC to lead (Pb) ranging from 750 to 1000 µg/ml and 600 to 750 µg/ml as compared to *E. coli*, *Klebsiella spp.* and *Staphylococcus aureus* which had lower MIC; ranging between 60 to 100, 60 to 150 and 50 to 100 µg/ml, respectively. Single isolate of *S. typhi*, *E. cloacae* and *Citrobacter spp.* showed MIC of 50, 270 and 672 µg/ml, respectively.

There was no change observed in morphology and biochemical reactions of the organisms after exposure of lead (Pb). Antibiotic susceptibility pattern was changed in all isolates after lead exposure. After exposure to lead, resistance to amoxicillin/clavulanic acid, co-trimoxazole, ceftazidime and amoxicillin/clavulanic acid was developed in *S. aureus* isolates. In *Enterobacteriaceae* family, third- and fourth-generation cephalosporins, gentamycin, ciprofloxacin, co-trimoxazole and tetracycline became resistant after lead exposure. Single isolate of *S. typhi* was sensitive to all tested antibiotics before exposure, while resistance developed to nalidixic acid, levofloxacin and ampicillin-sulbactam after exposure to lead (Pb). In *Pseudomonas spp.*, after lead exposure colistin, levofloxacin and cefepime showed resistance. In *Acinetobacter spp.*, resistance to cefepime, tetracycline, co-trimoxazole and ampicillin-sulbactam was reported after lead exposure. Change in antibiotic resistance was statistically significant for gentamycin, ciprofloxacin, ceftazidime, amoxicillin-clavulanic acid, tetracycline, co-trimoxazole and cefepime (*P* value < 0.05). Changes in resistance pattern of various antibiotics after lead exposure is shown in Chart 1.

The multiple antibiotics resistance (MAR) index was calculated among isolates before and after lead exposure by the formula given by Kawane *et al*^[1] According to that, MAR index for *S. aureus* was 0.2, *Klebsiella spp.*0.4, *E. coli* 0.4, *Acinetobacter spp.* 0.5, *Citrobacter spp.* 0.7, *Pseudomonas spp.* 0.6, *Proteus spp.* 0.3, *S. typhi* 0 and *E. cloacae* 0 before lead exposure. After lead exposure, MAR index for *S. aureus* was 0.4, *Klebsiella spp.*0.7, *E. coli*0.5, *Acinetobacter spp.* 0.6, *Citrobacter spp.* 0.9, *Pseudomonas spp.* 0.7, *Proteus spp.* 0.6, *S. typhi*0.2 and *E. cloacae* 0.3 as compared in Chart 2.

DISCUSSION

The present study deals with the change in antibiotic susceptibility pattern in clinical isolates after exposure to heavy metal (i.e. Pb). Resistance to number of antibiotics was increased after exposure of clinical bacterial isolates to lead. Thus, there was a significant change observed in multiple antibiotic resistance index in clinical isolates before and after lead exposure.

In a study by Kawane *et al.*^[1] 60 isolates of *E. coli* (30 from drinking water and 30 from clinical isolates) were included; antibiotic and heavy metal resistance patterns were studied by disc diffusion and cup method. The study revealed that 57% of clinical isolates had tolerance to heavy metal like Pb, Cd and Cu. Also, the antibiotic resistance noted in clinical isolates (64%) was more as compared to drinking water isolates (46%). The metal tolerance was 62% in water sample isolates and 57% in clinical isolates. According to the study, the incidence of high level of metal tolerance among bacteria was due to release of metal ions in water bodies due to geochemical processes. Nath *et al*^[11] have reported MIC of soil and sewage isolates to lead as 1400-1800 µg/ml for *Pseudomonas spp.*, 1600 µg/ml for *Klebsiella spp.* and 800 µg/ml for *S. aureus* and *Proteus spp.* each., which was more as compared to MIC of clinical isolates of the present study.

A study by Raja *et al*^[12] has also reported high degree of heavy metals resistance associated with multiple antibiotic resistances in sewage bacteria. In a study by Ug *et al.*^[13] 22 *Staphylococcus spp.* isolates recovered from clinical sources were studied for antibiotic

and heavy metal resistance patterns and plasmid profiles. The study has observed association between the occurrence of plasmids and resistance to antibiotics and heavy metals.

In antibiotic susceptibility, it was observed that aminoglycosides, fluoroquinolones, beta-lactam- lactamase combinations, cephalosporins, tetracycline, co-trimoxazole groups of antibiotics showed more resistance after lead exposure. In the MAR index, a change after lead exposure was noted significantly in *Enterobacteriaceae* family. The study by Nath *et al*^[11] has also reported more resistance to aminoglycosides, beta-lactams, tetracycline, cephalosporins, fluoroquinolones and co-trimoxazole after heavy metal exposure, though the isolates were from soil. The study has reported that multiple tolerances to antibiotics are common phenomenon among heavy metal-resistant isolates. The study by Atieno *et al*^[6] also has reported higher antibiotic resistance to tetracycline, chloramphenicol, co-trimoxazole and aminoglycosides after lead exposure in isolates from wastewaters of abattoirs. The study has shown that the combined expression of antibiotic and heavy metal resistance may not be a chance phenomenon but rather a result of selection by heavy metal presence in an environment.

The transfer of heavy metals and antibiotics from agriculture and animal husbandry to the environment may cause a combined effect of selection and co-selection toward antibiotic-resistant bacteria. It has been also reported that soil and water bodies impacted by agriculture and aquaculture act as hot spots for evolution of antibiotic-resistant bacteria which require special scientific consideration.^[14] It has been reported that even low levels of metal in soil and water may be associated with co-selection of antibiotic resistance in microorganisms.^[7] Therefore, getting infection with such antibiotic-resistant bacteria will lead us in post antibiotic era, where treatment of infectious disease would be difficult.

The limitation of the present study was that only one heavy metal was studied. Also, the numbers of clinical isolates studied were less. Further studies must be carried out with common heavy metals of the environment and change in antibiotic resistance after their exposure.

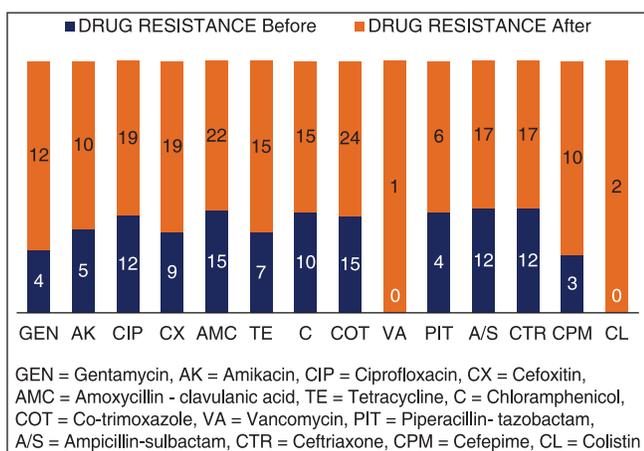


Chart 1: Change in antibiotics resistance after lead exposure

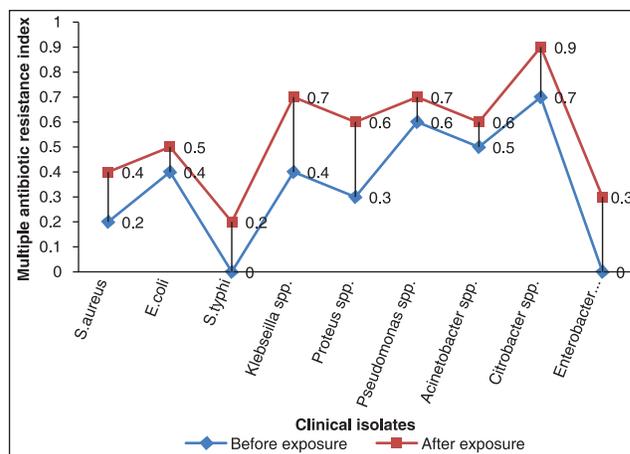


Chart 2: Change in multiple antibiotic resistance index after lead exposure in various clinical isolates

CONCLUSION

The MIC to heavy metal lead was higher in *Acinetobacter spp.* and *Pseudomonas spp.*, while that in *E. coli*, *Klebsiella spp.*, *S. typhi* and *Staphylococcus aureus* was less. The microorganisms like *Acinetobacter spp.*, *Pseudomonas spp.* and *Klebsiella spp.* are commonly associated with nosocomial infections. Therefore, higher level of drug resistance in them is of great concern. As heavy metal exposure greatly alter the antibiotic susceptibility of organisms, environmental exposure can further worsen the condition. Efforts to prevent environmental pollution with such heavy metals should be done by proper disposal of chemical waste generated from industries and healthcare setup.

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