International Journal of Life Sciences
International Peer Reviewed Open Access Journal

Special Issue A16, March 2021
ISSN:2320-7817(p) 2320-964X(0)

Original Article Open Access

Antibiogram of *E coli* isolates having heavy metals tolerance

Patil YS

Department of Microbiology, Shri D. M. Burungale College of Science and Arts, Shegaon-444203 Dist. Buldana, Maharashtra. India.
Emailid- yspsdmbsc@gmail.com

Manuscript details:

Available online on http://www.ijlsci.in
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Cite this article as:
Patil YS (2021) Antibiogram of *E coli* isolates having heavy metals tolerance, *Int. J. of. Life Sciences*, Special Issue, A16: 53-57.

Article published in Special issue of National Conference on "Recent Trends in Science and Technology-2021 (RTST-2021)" organized by Department of Environmental Science, Shri. Dnyaneshwar Maskuji Burungale Science & Arts College, Shegaon, Bhuldhana, and Department of Botany Indraraj Commerce and Science College Shillod, DIst. Aurangabad, Maharashtra, India date, February 22, 2021.

ABSTRACT

In the present Study total 10 heavy metal resistant *E. coli* were isolated from sewage of industrial effluents from waste water of Khamgaon M1DC area. Against copper, aluminium, iron, nickel and chromium. All the isolates exhibited high resistance to heavy metals with minimum inhibitory concentration for heavy metals ranging from 50 ug / ml to 500 ug / ml. All isolates showed multiple tolerance to heavy metal and were multi antibiotic resistant, heavy metal tolerance test indicated maximum microbial tolerance of *E. coli* to chromium (480 ug / ml) and lowest to Copper (60 ug / ml).

Keywords: Antibiotic resistance, heavy metal resistant bacteria, multiple tolerance.

INTRODUCTION

As the industrialization increases the pollution of the environment with toxic heavy metal also increases and spreads throughout the world. Copper, chromium, Aluminium, iron and nickel are known to be the most common heavy metals used and the more widespread contaminants of the environment (Patterson, 1977; Aksu, 1998; Doenmez and Aksu, 1999). The microorganisms respond to these heavy metals by several processes; including transport across the cell membrane, biosorption to the cell walls and entrapment in extracellular capsules, precipitation, complexation and oxidation reduction reactions (Rai *et al*., 1981; Macaskie and Dean, 1989; Huang *et al*., 1990; Avery and Tobin, 1993; Brady *et al*., 1994; Veglio *et al*., 1997). The bioremediation of heavy metals using microorganisms received attention in recent years, not only as a scientific novelty but also for its potential application in industry. Metal accumulative bioprocess generally falls into one of two categories, bisorptive (passive) uptake by nonliving, non growing biomass or biomass products and bioaccumulation by living cells (Macaski and Dean, 1989; Aksu and Kutsal, 1990; Huang *et al*., 1990; Volesky *et al*., 1992; Avery and Tobin, 1993; Brady and Duncan, 1994; Aksu, 1998; Doenmez and Aksu, 1999; 2001).

Industrial wastes containing toxic metals were characterized by their differences rather than their similarities. These toxic metals can arise from a wide variety of industrial processes. The quality and the quantity of the wastes containing toxic heavy metals are dependent upon their industrial sources. Naturally occurring bacteria that are capable of metal accumulation have been extensively studied since it is difficult to imagine that a single bacterium could be capable to remove all heavy metals from its polluted site (Clausen, 2000). Apparently, the metal, which has been introduced into the bacterial suspension by vigorous mixing, forms complexes with various ligands available (Hussein *et al*., 1998; 2001). Consequently, the largest number of metals will be found as hydroxide or as a stable metal-ligand complex. Under a specific stress conditions, a relatively constant amount of metal reacts to stable and inactive complexes with active cellular components (Hussein, 1999). However, it is very important before the optimization of the bacterial growth process is to study at which pH value will be found as metal ions to study the exact interaction between the free metal ions and the bacterial strain. This study aimed to isolate and characterization of *E. coli* sp resistant to heavy metals contaminants from sewage of industrial effluents from waste water of Khamgaon M1DC area.

MATERIAL & METHODS

Sample collection

The sampling area was the sewage of industrial effluents from waste water of Khamgaon M1DC area. Samples was collected in sterile plastic bottles. A total of twenty samples were taken for study.

Isolation and identification of bacterial heavy metal resistance

For the selective isolation of heavy metals resistant bacteria, media incorporated with heavy metals were used. The Nutrient agar incorporated with heavy metals like $Cr6^{+}$, $Ni2^{+}$, $Al2^{+}$, $Fe2^{+}$ and $Cu2^{+}$ were prepared. The concentration of each heavy metal was maintained at 50 µg/ml of the medium. The wastewater sample directly streaked on (N. A.) media and incubated at 37°C for 24 h. After the incubation period the plates were observed for any kind of growth on the media. The isolated colonies of *E. coli* sp. on the Nutrient agar media were sub cultured and obtain in the form of pure culture and identified on the basis of their morphology and biochemical characters.

Determination of Minimum Inhibitory Concentration (MIC)

MIC of the heavy metal resistant *E. coli* sp. grown on heavy metals incorporated media, against respective heavy metal was determined by gradually increasing the concentration of the heavy metal, 10 µg/ml each time on Nutrient agar plate until the strains failed to give colonies on the plate. The starting concentration used was 50µg/ml. The culture growing on the last concentration was transferred to the higher concentration by streaking on the plate. MIC was recorded when the isolates failed to grow.

Determination of antibiotic sensitivity and resistance pattern

Antibiotic sensitivity and resistance of the isolated heavy metal resistant isolates were assayed according to the Kirby-Bauer disc diffusion method given by Bauer *et al*. (1996). On completing incubation, the organisms were classified as sensitive or resistant to given antibiotic according to the zone diameter showing inhibition given in standard antibiotic disc chart.

RESULTS & DISCUSSION

Isolation and identification of bacterial heavy metals resistance

Ten heavy metal resistant *E. coli* were isolated from, sewage of industrial effluents from waste water of Khamgaon M1DC area. against chromium, copper, nickel, aluminium, iron. All the isolates exhibited high resistance to heavy metals with minimum inhibitory concentration (MIC) for heavy metals ranging from 50µg/ml to 500µg/ml. All isolates showed multiple resistance to heavy metal and were multi antibiotic resistant. Heavy Metal Tolerance Test indicated highest tolerance to Chromium (480µg/ml) by Ec -5 isolates and lowest to Copper (60µg/ml) by Ec 7

Antibiotic sensitivity of heavy metals resistant isolates

All the isolates were resistant to antibiotics of which EC-7 was found to be single antibiotic resistance while the rest of the isolates were found to be multi-antibiotic resistant (Table 2).

The sewage of industrial effluents from waste water of Khamgaon MIDC area, the domestic as well as industrial wastewater from Khamgaon region were collected.

Table 1. Resistance of bacteria to other heavy metals. (Ec= *E. coli* isolates).

Bacteria	Resistance to	MIC
Ec -1	Al^{+2}	130 µg / ml
	Cr^{+6}	180 µg / ml
	Cu^{+2}	120 µg / ml
	Fe^{+2}	180 µg / ml
	Ni^{+2}	140 µg / ml
Ec -2	Al^{+2}	120 µg / ml
	Cr^{+6}	180 µg / ml
	Cu^{+2}	120 µg / ml
	Fe^{+2}	125 µg / ml
	Ni^{+2}	130 µg / ml
Ec -3	Al^{+2}	140 µg / ml
	Cr^{+6}	225 µg / ml
	Cu^{+2}	80 µg / ml
	Fe^{+2}	80 µg / ml
	Ni^{+2}	110 µg / ml
Ec -4	Al^{+2}	180 µg / ml
	Cr^{+6}	90 µg / ml
	Cu^{+2}	140 µg / ml
	Fe^{+2}	110 µg / ml
	Ni^{+2}	120 µg / ml
Ec -5	Al^{+2}	120 µg / ml
	Cr^{+6}	480 µg / ml
	Cu^{+2}	150 µg / ml
	Fe^{+2}	80 µg / ml
	Ni^{+2}	280 µg / ml
Ec -6	Al^{+2}	70 µg / ml
	Cr^{+6}	300 µg / ml
	Cu^{+2}	120 µg / ml
	Fe^{+2}	200 µg / ml
	Ni^{+2}	180 µg / ml
Ec -7	Al^{+2}	400 µg / ml
	Cr^{+6}	180 µg / ml
	Cu^{+2}	60 µg / ml
	Fe^{+2}	100 µg / ml
	Ni^{+2}	120 µg / ml
Ec -8	Al^{+2}	60 µg / ml
	Cr^{+6}	210 µg / ml
	Cu^{+2}	120 µg / ml
	Fe^{+2}	250 µg / ml
	Ni^{+2}	140 µg / ml
Ec -9	Al^{+2}	120 µg / ml
	Cr^{+6}	240 µg / ml
	Cu^{+2}	200 µg / ml
	Fe^{+2}	70 µg / ml
	Ni^{+2}	340 µg / ml
Ec -10	Al^{+2}	130 µg / ml
	Cr^{+6}	180 µg / ml
	Cu^{+2}	120 µg / ml
	Fe^{+2}	180 µg / ml
	Ni^{+2}	140 µg / ml

Table 2. Antibiotic sensitivity and resistant activity of heavy metal resistant *E. coli* sp

Sr.No.	Bacteria	Sensitive to	Resit ant to
1	Ec -1	Bacitracin, Vancomycin, Chloramphenicol, Erythromycin, Tetracycline, Amikacin and kanamycin	Ampicillin, Ciprofloxacin, Tetracycline,
2	Ec -2	Ampicillin, Ciprofloxacin, Chloramphenicol, Erythromycin, Amikacin and kanamycin	Tetracycline, Bacitracin, Vancomycin,
3	Ec -3	Ampicillin, Bacitracin, Vancomycin, Chloramphenicol, Erythromycin, Tetracycline and Amikacin	Ciprofloxacin, and kanamycin
4	Ec -4	Ampicillin, Ciprofloxacin, Bacitracin, Vancomycin and kanamycin	Chloramphenicol, Erythromycin, Tetracycline, and Amikacin
5	Ec -5	Ciprofloxacin, Bacitracin, Vancomycin, Chloramphenicol and Erythromycin	Ampicillin, Tetracycline, Amikacin and kanamycin
6	Ec -6	Ciprofloxacin, Tetracycline Bacitracin, Tetracycline, Amikacin and kanamycin	Ampicillin, Vancomycin, Chloramphenicol, Erythromycin
7	Ec -7	Ampicillin, Ciprofloxacin, Vancomycin, Chloramphenicol, Erythromycin, Amikacin and kanamycin	Tetracycline,
8	Ec -8	Ampicillin, kanamycin, Ciprofloxacin, Tetracycline, Bacitracin, Vancomycin, Chloramphenicol, Erythromycin, Tetracycline, Amikacin	Ampicillin, Vancomycin, Chloramphenicol, Erythromycin
9	Ec -9	Ampicillin, Bacitracin, Vancomycin, Chloramphenicol, Erythromycin, Tetracycline and Amikacin	Ciprofloxacin, and kanamycin
10	Ec -10	Ciprofloxacin, Tetracycline Bacitracin, Tetracycline, Amikacin.	Ampicillin Erythromycin

The wastewater coming from domestic and industrial sources is the appropriate environment where the microorganisms can develop resistance to heavy metals and antibiotics. The presence of small amount of antibiotics and heavy metals in the wastewater induce the emergence of Antibiotic resistance of heavy metal resistant isolates antibiotic resistance and heavy metal resistant microorganisms. Most of the isolates in the present study showed multiple tolerances to both heavy metals and antibiotics. The microbial resistance to heavy metal is attributed to a variety of detoxifying mechanism developed by resistant microorganisms such as complex exopolysaccharides, binding with bacterial cell envelopes, metal reduction, metal efflux etc. These mechanisms are sometime encoded in plasmid genes facilitating the transfer of toxic metal resistance from one cell to another (Silver,1996). Filali *et al*. (1999) studied wastewater bacteria isolates *Psuedomonas aeroginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Staphylococcus* resistant to heavy metals and antibiotics. Similarly, Sharma *et al*. (2000) isolated highly cadmium resistant Klebsiella that was found to precipitate significant amount of Cds. The heavy metal resistant organism could be a potential agent for bioremediation of heavy metals pollution. Multiple tolerances occur only to toxic compounds that have similar mechanisms underlying their toxicity. Since heavy metals are all similar in their toxic mechanism, multiple tolerances are common phenomena among heavy metal resistant bacteria. In wastewater, there are some substances that have the potential to select for antibiotic resistance even though they are not antibiotics themselves. Heavy metals and biocides are two of them. The exposure to heavy metals or biocides results in the selection of bacterial strain also able to resist antibiotics. This happens because genes encoding heavy metals and biocides are located together with antibiotic resistance genes or alternatively because bacteria can have unspecific mechanism of resistance common to different substances including heavy metals, biocides and antibiotics (Dalsgarrd and Guardbassi, 2002). It is therefore, likely that selective pressure by one such compound indirectly selects for the whole set of resistances.

Conflicts of interest: The authors stated that no conflicts of interest.

REFERENCES

Aksu Z (1998). Biosorption of heavy metals by microalgae in batch and continuous systems. In: Algae for waste water treatment. eds Y.-S. Wong and N. F. Y. Tam, 99.37-53. Springer, Germany.

Aksu Z and Kutsal T (1990). Acomparative study for biosorption characteristics of heavy metal ions with C. vulgaris. Environ. Technol., 11: 979-987.

Avery SV and Tobin JM (1993). Mechanism of adsorption of hard and soft metal ions to Saccaromyces cerevisiae and influence of hard and soft anions. Appl. Environ. Microbiol, 59: 2851-2856.

Brady D and Duncan JR (1994). Chemical and enzymatic extraction of heavy metal binding polymers from isolated cell walls of Sccharomyces cerevisiae. Biotechnol. Bioeng., 44: 297-302.

Brady D, Stoll AD, Starke L and Duncan JR (1994). Bioaccumulation of metal cations by Saccaromyces cerevisiae. Appl. Microbiol. Biotechnol., 41: 149-154.

Clausen CA (2000). Isolating metal-tolerant bacteria capable of removing copper, chromium, and arsenic from treated wood. Waste Manage Res., 18: 264-268.

Doenmez G and Aksu Z (1999). The effect of copper(II) ions on the growth and bioaccumulation properties of some yeasts. Process Biochem., 35: 135-142.

Dalsgarrd A and L Guardbassi (2002). Occurrence and fate of antibiotics resistant bacteria in sewage. Danish, EPA Environment Project No. 722.

Doenmez G and Aksu Z (2001). Bioaccumulation of copper(II) and Nickel (II)m by the non adapted and adapted growing Candida sp. Wat. Res., 35: 1425-1434.

Filali BK, J Taoufik, Y Zeroual, FAZ Dzairi, M Talbi and M Blaghen (1999). Waste water bacterial isolates resistant to heavy metals and antibiotics.Current Microbiology 41: 151-156.

Huang C, Huang C and Morehart AL (1990). The removal of copper from dilute aqueous solutions by Saccharomyces cerevisiae. Wat. Res., 24: 433-439.

Hussein H, Krull R, Abou-ElEla SI and Hempel DC (1998). Influence of heavy metal ions on the microbial degradation of xenobiotic waste water compounds, AWT98-Advanced wastewater treatment, recycling and reuse, Milano, 14-16 September.

Hussein H, Krull R, Abou-ElEla SI and Hempel DC (2001). Interaction of the different heavy metal ions with immobilised bacterial culture degrading xenobiotic waste water compounds. Second International Water Asociation World Water Conference (2nd IWA) Berlin, 15-19 October.

Koedam N, Wittouck E, Gaballa A, Gillis A, Hfte M and Cornelis P (1994): Detection differentiation of microbial siderophores by isoelectric focusing and chrome azurol S overlay. Biometals, 7: 287- Krieg, N.K. (Ed.) (1984). Bergey`s manual of systematic bacteriology. Baltimore, Hong Kong, London, and sydney, 1: 178-182.

Macaskie L and Dean ACR (1989). Microbial metabolism, desolubilisation and deposition of heavy metals: Metal uptake by immobilised cells and application to the detoxification of liquid wastes. Adv. Biotechnol. Proc., 12: 159-172.

Patterson JW (1977). Wastewater treatment technology. Ann Arbor science Publishers,Ann Arbor, MI, USA.

Rai LC, Gaur JP and Kumar HD (1981). Phycology and heavy metal pollution. Biol. Rev., 56: 99-151.

Silver S (1996). Bacterial resistances to toxic metal ions-a review. Gene 179: 9-19.

Sharma KP, A Frenkel and LD Balkwill (2000). A new Klebsiella planticola strain (cd-1) grows anaerobically at high cadmium concentrations and precipitates cadmium sulphide. Applied and Environmental Microbiology 66: 3083-3087.

Veglio F, Beolchini F and Gasbarro A (1997). Biosorption of toxic heavy metals: an equilibrium study using free cells of Arthrobacter sp. Process Biochem., 32: 99-105.

Volesky B., May H and Holan ZR (1992).Cadmium biosorption by Saccharomyces cerevisiae. Biotechnol. Bioeng., 41: 826-829.