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Shahla Mansouri
Department of Microbiology
Kerman University of
Medical Sciences
P.O. Box 444
Kerman, Iran

Tel: +98-341-3221660-2
Fax: +98-341-3221671

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Screening 469 *Escherichia coli* Isolates Form South East of Iran for Tellurite Resistance Phenotype

[1]Shahla Mansouri and [2]Seyd Mohammad Nayebeeaghaei

Minimum Inhibitory Concentration (MIC) of tellurite was determined for 469 samples of *Escherichia coli* isolated from clinical samples in human (urinary tract infection, normal fecal flora), or poultry skin using standard agar dilution method. About half of the isolates (47.1%) had MIC of ≤1 μg mL^{-1} after 24 h of incubation at 35°C. Many of the bacteria that were cultured with low concentrations of potassium tellurite, ≤ 5 μg mL^{-1}, overcome the toxicity of this heavy metal when they were re-incubated for the next 24 h. The MIC for these isolates was increased, so that the difference between the MIC after 24 and 48 h of incubation became significant ($p \leq 0.0026$). In medium containing ≥10 μg mL^{-1} of potassium tellurite, the bacteria formed typical black colonies. No statistically significant difference was found between the MIC of bacteria that where isolated from human, but higher rate of resistance was found for the Poultry skin samples and 25% of these isolates had MIC of ≥160 μg mL^{-1}. Although for nearly half of the isolates potassium tellurite was toxic at low concentration (≤1 μg mL^{-1}), higher MIC was observed for the rest of isolates (10-400 μg mL^{-1}) and is an indication of the widespread resistance to potassium tellurite in *E. coli* in this region.

Key words: Tellurite resistance, *Escherichia coli*, urinary tract infections, fecal flora, poultry

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[1]Department of Microbiology, Kerman University of Medical Sciences, P.O. Box 444, Kerman, Iran
[2]University of Lorastan, Lorastan, Iran

INTRODUCTION

Heavy metals are used in a variety of industrial applications and are potential source of environmental contamination[1]. Many metals are essential component of the cell at low level, but can exert toxic effects at high concentration such as those encountered in polluted environments[2]. According to Sanchez-Romero *et al.*[3] tellurite resistance is an attractive marker for genetic manipulations of microorganisms and spontaneous tolerance to it is reported to be very infrequent and resistance determinants are rarely expressed. But Alonso *et al.*[4] reported the presence of plasmids of H incompatibility complex in *Escherichia coli*, which confers resistance to potassium tellurite and all known channel-forming colicins and suggested that presence of this character confers selective advantage on cells sharing an ecological niche. Presence of plasmids which confers resistance to potassium tellurite in *E. coli* from human sources and their wide distribution among enterobacteria in nature has also been reported by Whelan *et al.*[5]. This is not surprising because bacteria have been so exposed to heavy metal contamination in the environments that they have developed genetically resistance systems against heavy metal toxicity such as extra cellular precipitation and enzymatic oxidation or reduction to a less toxic form[2,6,7]. Toxicity of tellurite is believed to be from its oxidative activity and therefore it has a broad spectrum of activity against many microorganisms[1,8]. One possible mechanism to detoxify tellurite is to reduce it to metallic tellurium (Te) and it has been shown that bacteria grown on media containing potassium tellurite form black colonies due to the intracellular deposition of tellurium[1,7,9].

Tellurite has been used for more than 80 years in selective media for the isolation of pathogens including *Corynebacterium diphtheria*, *Staphylococcus aureus* and *Shigella* sp.[10]. It has also been used in many media designed for the isolation of vero-cytotoxigenic *E. coli*[10-12] and higher MIC for these isolates in comparison with other strains of *E. coli* has been reported by Zadik *et al.*[12].

Most of the recent research on tellurite resistance in bacteria are on standard or specially cloned strains, in the present study we report the MIC for 469 wild type *E. coli*, isolated from Urinary Tract Infections (UTI) and Fecal Flora (FF) from human subjects and from Poultry Skins samples (PS).

MATERIALS AND METHODS

A total of 469 *E. coli* (UTI: 265, FF: 184 and PS: 20) were used in this study. The samples were isolated during March to July 2000 in Kerman (southeast of Iran). For the isolation of bacteria from poultry, samples from chicken skin were obtained randomly from local poultry shops. These samples were suspended in sterile saline solution (dilution 1/10 W/V) and were kept for 24 h at 4°C, after which it was mixed and supernatant (100 μL) was cultured on EMB agar. All the bacterial isolates were identified by standard biochemical tests[13] and were kept on trypticase soy broth with 40% glycerol at -70°C.

MIC of the isolates against Potassium tellurite was tested using standard agar dilution method[14]. All tests were performed on Muller-Hinton agar (Oxoid Co, Hampshire, UK). Suspension of the bacteria (10 μL) containing $1x10^4$ organisms ($1x10^6$ organisms mL^{-1}) was inoculated in the media with different concentrations of filter sterilized Potassium tellurite (E. Merck., Germany). MIC was recorded when the number of growing colonies in the agar was ≤5.

E. coli strain ATCC 25922 and ATCC 8793 were used as the quality control strains. EPI Info, version 6, was used as a word-processing data-base and statistics program for public health-analyzed data.

RESULTS

Minimum Inhibitory Concentration (MIC) of 469 *E. coli* isolates on Muller-Hinton agar was recorded 24 and 48 h after incubation at 35°C. The MIC of 1 μg mL^{-1} was found for 221 (47.1%) of the isolates after 24 h of incubation. Many isolates that were cultured in low concentrations of potassium tellurite (up to 5 μg mL^{-1}) begin to grow when the plates were re-incubated for the next 24 h and the difference in the MIC of these isolates at 24 and 48 h of incubation became statistically significant (Table 1). Standard strains, ATCC 25922 and ATCC 8793 grow only on the media containing 0.5 μg mL^{-1} Potassium tellurite after 24 h of incubation at 35°C. After re-incubation for another 24 h, strain ATCC 25922 was able to grow on the agar medium containing 0.5, 1 and 2.5 μg mL^{-1} potassium tellurite, but the strain ATCC 8793 grows only on the concentrations of 0.5 μg mL^{-1} potassium tellurite (results not shown). All the bacteria that were able to grow on the medium containing ≥10 μg mL^{-1} of potassium tellurite formed typical black colonies. Frequency of tellurite resistance MIC for the bacteria that were isolated from urinary tract infection and fecal samples were approximately similar with no statistically significant difference (Table 2). However higher rate of resistance to potassium tellurite was found for the bacteria that were isolated from the chicken skin and 35% of these isolates were resistance to high level of Potassium tellurite (≥40 μg mL^{-1}) in the medium and one strain was resistance to >400 μg mL^{-1} of potassium tellurite in the medium (results not shown).

Table 1: MIC of Potassium tellurite for 469 isolates of *E. coli* 24 and 48 h after incubation at 35°C

Incubation	MIC μg mL^{-1} [Number (%)]								
Time (h)	1	2.5	5	10	20	40	80	≥160	Total
24	221(47.1)	31(7.3)	18(3.8)	45(9.2)	65(13.6)	64(14.3)	13(2.6)	12(2.1)	469(100)
48	169(36.0)	58(12.4)	47(10)	52(11.1)	59(12.6)	56(11.9)	16(3.8)	12(2.1)	469(100)
p-value	0.00057	0.0026	0.00019	0.45	0.56	0.43	0.57	1.0	-

The number of isolates from urinary tract infections, fecal flora, or poultry samples were 265,184 and 20, respectively

Table 2: Comparison between MIC values for Potassium tellurite in *E. coli* isolated from Urinary Tract Infection (UTI), fecal flora or poultry samples

Samples	MIC μg mL^{-1} Number (%)								
	1	2.5	5	10	20	40	80	≤160	Total
Fecal flora	94(51.08)	9(4.89)	7(3.8)	17(9.24)	21(11.41)	27(14.67)	6(3.26)	3(1.6)	184(100)
Urinary tract	125(47.17)	19(2.26)	9(3.4)	25(9.40)	41(15.47)	36(13.58)	6(2.26)	4(1.5)	265(100)
Poultry	2(10.00)	3(15)	2(10)	3(15.0)	3(15.00)	1(5.00)	1(5.00)	5(25.0)	20(100)
*p-value	0.4(0.0056)	0.32(0.19)	0.81(0.33)	0.94(0.7)	0.22(0.46)	0.74(0.48)	0.52(0.67)	0.91(0.0000)	-

* p-value: Difference between MIC of the samples isolated from urinary tract infection with that of fecal flora, (p-value): For the difference between the MIC of the three samples

DISCUSSION

Studying metal ion resistance gives us an important insight into environmental processes and provides an understanding of basic living processes. There are controversy regarding the resistance mechanism and MIC of tellurite in *E. coli*, Taylor *et al.*[9] reported that resistance to potassium tellurite is rare in bacteria and Tomas and Key[15] found that potassium tellurite is very toxic towards *E. coli* with the MIC of approximate 1 μg mL^{-1}. In the present study for about half of the isolates potassium tellurite was found to be very toxic with an MIC of ≤1 μg mL^{-1} (Table 1). No difference was found between the resistance to this metal ion in the UTI or FF, but for the isolates from PS higher rate of resistance was found and 25% of these isolates had MIC of ≥160 μg mL^{-1}. Hiramatsu *et al.*[16]; Fukushima *et al.*[11] and Taylor *et al.*[10] reported that majority of Shiga toxin producing *E. coli* (STEC) have a higher MIC than non STEC and MIC of these strains were in a range of 25-200 mg mL^{-1}. In the present study there were 7 strains which were identified to be *E. coli* O157:H7 by vero cell cytotoxicity and agglutination with O:157 antiserum (unpublished results). The MIC for these isolates ranged from 2.5 to 20 mg mL^{-1}, which is much lower than those reported by other workers. We tried to do the agar dilution method under complete standard condition, since we found that slight difference in the inoculums size can greatly affect the consistency of the results, so the discrepancy in the reported MIC could be due to this factor, for example Taylor *et al.*[10] used a higher number of cells for spotting the plates with potassium tellurite. Besides inoculum size several other factors can affect the toxicity of heavy metal ions, such as constitution of the medium and presence phosphate, which can protects the cell against the toxic effect of a range of metals[6].

It was interesting to note that the bacteria somehow overcome the toxic effect of low concentration of potassium tellurite in the medium (Table 1). Same results were found in the case of *Saccharomyces cerevisiae* with vanadate and with *E. coli* K_{12} and cadmium. For vanadte, it is hypothesized that at low concentration this ion is reduced to vanadyl and is excreted in this form, but at higher concentration the rate of vanadate influx overtakes that of vanadate reduction, therefore a toxic high molecular mass vanadte will form and stops growth[17]. However in case of cadmium at low concentration, the period of stasis is required for the cell to repair the damage and adjust the cell physiology to limit the distribution of this toxic ion in the cell[18]. There is no such report for potassium tellurite and further work is needed to clarify this effect.

Although the number of isolates from chicken skin was low in present study, high level of resistance in these isolates is important, since many of the resistance isolates may be originated from food. In present study using a large number of wild type *E. coli* isolates, we found that resistance to tellurite is not a special character of certain isolates such as O:157 isolates, it is present in clinical as well as environmental samples and is widespread in this bacterium in the region. However many other questions such as precise mechanism of resistance to tellurite and other unrecognized functions associated with resistance to tellurite is still to be answered.

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