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Original Research Article

Synergistic effects of the bismuth nanoparticles along with antibiotics on *PKS* positive *Klebsiella pneumoniae* isolates from colorectal cancer patients; comparison with quinolone antibiotics

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Abstract

Colorectal cancers (CRC) are among the most prevalent types of cancers in the world and in Iran, wherein, the age of affliction is lower than Western countries. We studied the antimicrobial effect of the bismuth nanoparticles on *Klebsiella pneumoniae* carrying *PKS* gene. *PKS* encodes colibactin which makes colorectal cancers more probable. In the present study we identified and isolated the *PKS* carrier isolates through application of PCR. Subsequently, the antibacterial effects of the bismuth nanoparticles, along with antibiotics including tetracycline; ciprofloxacin, Norofloxacin, and metronidazole were investigated on bacterial isolates. Results show all isolates were sensitive to all applied antibiotics, however, they showed resistance to the bismuth nanoparticles. Analysis on the synergistic effect of the bismuth nanoparticles with the aforementioned antibiotics shows a synergistic effect, however, there was difference between bacterial isolates as well as the degree of synergism. Results obtained in this study indicate, compared to the antibiotics, the bismuth nanoparticles could be effective against digestive system's infection to lower extents. As well, it could be effective in lowering the colorectal cancer in the individuals' carrier for the isolates.

Keywords: Bismuth naoparticles, colibactin, colorectal cancer, *Klebsiella pneumoniae*, *PKS*

INTRODUCTION

As a human natural microbial flora, *Klebsiella pneumoniae*, a Gram negative bacterium and member of *Enterobacteriaceae* composes a part of intestinal microflora in one third of the healthy individuals. *K. pneumoniae* is now considered as opportunistic important infective clinical pathogens that result in the high rate of the mortality and involvement in many urinary tract infections, septicemia, pneumonia, and the bowl infections in the hospitalized patients. In non hospitalized individuals there is less involvement for *K. pneumoniae* (Padilla E, liobet E, 2010). Microbial resistant is a serious problem that affects health throughout the world and in this regards resistance to the antibiotics in the intestinal microbial flora provide an opportunity for fungal and opportunistic bacterial invasions (Fernandez A,Pereira MJ, 2011, Jean and Hsueh, 2011). The infectability of the *K. pneumoniae* depends on the reduced host defense as a result of different types of drugs and operation, the two factors that result in weakening of the host defense system. Therefore, the increased resistance to antibiotics necessitates a better understanding of the antibacterial resistance mechanism against a specific antibiotic in order to treat the pathogen (Gangoue PJ, Ngassam P, 2006). This notion is especially important regarding the increased antibiotic resistance in the communities against a wide spectrum of the antibiotics that require new strate-

gies for a better treatment. Studies have shown that several strains of the *Klebsiella* produce a protein called colibactin that is involved in DNA strand breaks and an increased colorectal cancers (CRC) (Cuevas-Ramos G, Petit CR, 2010).

It is suggested that application of antibacterial agents with the wider spectrum could prevent colorectal cancers and as a candidate; bismuth nanoparticles, as we have used in the present study against *PKS* carrier *K. pneumoniae* isolated from CRC patients.

Bismuth is a fragile crystalline metal, and naturally, with a very high magnetism property. Bismuth is usually present in three forms; bismuthinite (bismuth sulfide), bismuth oxide, and bismuth carbonate (Drummond DC, 2006). The metal expands when it is frozen and shows high electrical conductivity, while, its thermal conductance is lower than every type of metals except mercury (Briand and Burford, 1999). Among the three above kinds of bismuth the bismuth oxide has received much technological importance. In general bismuth salts are used as precursors for surface modification, and production of nanoparticles. The properties of the nanoparticles are characterized through analysis of the bismuth colloids using X-ray and high resolution transmission electron microscope (HR-TEM) (Velasco-Arias D, 2012). This method is common for nanoparticle synthesis which is cheap and could be carried out at industrial scale. Bismuth salts are commonly applied in gastric and intestinal disorders. While bismuth has antimicrobial effects, however, due to its low water solubility it is required that a high concentration of which to be applied for treatment. Chelation increases the solubility as well as the antimicrobial effects of the bismuth. For example bismuth dimercaptopropanol (Bis-BAL) is used at lower concentrations (Domenico P, 1997). However, in a long time period the effect of Bis-BAL will decline. Application of bismuth in form of nanoparticles causes its slow release and could have positive impact and duration of the antimicrobial effect for long time.

Bismuth salts that still are used in gastric and intestinal disorders are pitobismol (bismuth subsilate; BSS) (Figueroa-Quintanilla D,Salaxar-Lindo E, 1993), De-Nol (colloidal bismuth subsalicylate; CBS) and derivatives of CBS such as ranitidine bismuth citrate (RBC) (Briand and Burfard, 1999). Bismuth compounds are used for syphilis (Willcox, 1999), and tumor treatment (Kopf-Maier P, 1994), as well as application in the radioisotope treatment plus many other applications such as biomolecules identification and as a wide spectrum antimicrobial agent (Lein and Chang, 2012).

Bis-NPS show antiviral, antifungal, and bactericidal property. It was suggested that Bis-NPS is not toxic for human cells. Exposure of the monkey kidney cells to 2µM nanobismuth particle for 34 hours didn't show any toxic effects (Hernandez-Delgadillo R,2012). Bismuth nanoparticles are new achievement for infectious diseases treatment; however, more experiments are required before prescription to human. For example, while silver nanoparticles are effective antibacterial agents, however, several reports indicate for their toxic effects (Yamanaka M,Hara K, 2005). The cytotoxic effect of bismuth oxide nanoparticle (BONPS) was studied on *Cepa allium* (onion). It was found that BONPS has genotoxic effects on root meristem cell (Liman, 2013). These observations necessitate further investigations before any application of the bismuth nanoparticle in the treatment of human diseases.

Colibactin is a bacterial protein product which induces DNA double strand breaks (DDRs) in cells along with an increased H2AX levels (Cuevas-Ramos G, Petit CR, 2010). DDRs are necessary for preservation and integrity of genome in the cells exposed to the endogenous reactive oxygen species (ROS) produced during metabolism as well as environmental factors (e.g. ionizing radiations and UV, etc.).

Activation and assay of DDR happens along with the phosphatidyl inositol kinase 3, in addition to its homologue kinases such as ATM, ATR, and DAN-PK. The end result will be inhibition of the cell cycle and activation of DNA repair system. When repair is completely done then cell cycle returns back to its normal condition, otherwise, the cell undergoes apoptosis and senesce. These events also result in the genomic instability (Podhorecka M, Skladanowski A, 2006). p53 is the key tumor suppressor protein in controlling and regulating cell death and senescence in the unrepaired DNA damaged cells. DSBs are repaired through non homologus end joining or non-homologous recombination in the cell cycle. During strand break and joining that occurs during this type of repair addition or deletion of nucleotide may occur. In contrast homologous repair (HR) is error free because sister chromatids are used as templates. HR is limited to the late G2 and S phases of the cell cycle.

METHODS

Bismuth nanoparticles were prepared from purchased bismuth sulfide salt. The structure and quality of the nanoparticles were analyzed using scanning electron microscope (SEM) and was used at a concentration of 3500 ppm. Briefly 5cc of the bismuth nanoparticles at 3500 ppm were transferred to a tube in sterile condition. To this mixture, sterile water was added to obtain 20 cc vismuth nanoparticles with specific concentration as calculated using the simple formula: M1V1=M2V2.

Antibiotic and bismuth subcitrate (BSC) to a specific concentration in mg were prepared in the sterile water as solvent and mixed well.

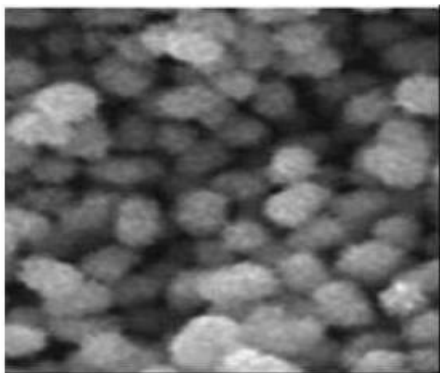

Figure 1. Shape of bismuth nanoparticles as identified using scanning electron microscope (SEM). Particles are spherical in shape with irregular walls and 170 nm up to 200 nm size.

Polymerase Chain Reaction (PCR)

DNA was extracted using DNA extraction kit (Cinnagen) according to the manufacturer's instruction and was checked through running on 0.8% agarose gel. 1µl of the extracted DNA, whose concentration was measured using spectrometer at 260 nm, was mixed with 12.5 1µl master mix containing Taq DNA polymerase plus 9.5 1µl double distilled water and 0.5µl (10 pmole) of the forward and reverse primers. The mixture was mixed well and amplification cycles was done according to the following program: 95 °C denaturation 5 min, 56.3 °C annealing 30 sec, 72 °C extension 30 sec for 35 cycles plus the final extension at 72 °C for 10 min. PCR products were run on 2 % agarose gel, stained with ethidium bromide and visualized using UV illuminator.

The sequences of primers were as follows:

5'GAT TTG GAT ACT GGC GAT AAC CG 3' ClbB Forward

5'CCA TTT CCC GTT TGA GCA CAC 3' ClbB Reverse

5'GTT TTG CTC GCC AGA TAG TCA TTC 3' ClbN Forward

5'CAG TTC GGG TAT GTG TGG AAG G 3' ClbN Reverse

The Gram negative microbial isolates were cultivated on Muller Hinton agar, incubated for 24h at 37 °C and staining was done using Gram stain. Whenever it was necessary, a suspension culture of microbial isolates was prepared using a colony of grown microbe on agar plate in 10 ml physiological serum with 0.9% NaCl. When turbidity of the cultured reached to 0.1 at 600 nm which accounts for 10^8 CFU/ml 1 ml of which was mixed with 9 ml physiological serum (10^7 CFU/ml), mixed well, and was used for inoculation in subsequent experiments for minimal inhibitory concentration (MIC) test and minimal bactericidal concentration (MBC) effect of bismuth nanoparticles in Muller Hinton broth. Serial dilution of bismuth nanoparticles were prepared starting with 1750 ppm concentration. 13 serial dilutions, each of which, was composed of the half amount of the bismuth nanoparticle in ppm were prepared, 100 µl of bacterial culture was added to each tube, vortexed and incubated for 24 h at 37 °C. Subsequently the turbidity of the tubes was measured as above and the lowest concentration (as ppm) wherein there wasn't any growth was considered as MBC (Wei SW,Q ian W, 2009). For antibiotics we used the same procedure starting with 2048 µg/ml in sterile distilled water and subsequently the MIC and MBC were measured. In addition the synergistic effect of bismuth nanoparticles and antibiotic was also investigated starting with the 1750 ppm/ml bismuth nanoparticles plus 2048 µg/ml of the antibiotic.

Disk diffusion test was done using 10^8 CFU on Mueller Hinton agar plates using the following disks containing antibiotics ciprofloxacin, Norofloxacin, tetracycline**,** and metronidazole. BSC pills (120 mg) and bismuth nanoparticle solution (3500 ppm) were used in order to investigate the effect of bismuth nanoparticles. Regarding bismuth nanoparticle 30 µl of solution was applied onto the wells that were made on agar plate of bacterial culture. The growth inhibitory area was measured in mm subsequently.

Challenge test was also done applying 10^8 CFU/ml of the bacteria on Muller Hinton agar plates using antibiotics and bismuth nanoparticles as explained for measurement of MIC and MBC. All experiments were repeated at least three times and the mean of bacterial growth was calculated or zone of inhibition was determined.

RESULTS

Using scanning electron microscope, the size of the bismuth nanoparticles was found to be 170 – 200 nm with a spherical shape and irregular outer wall (figure 1).

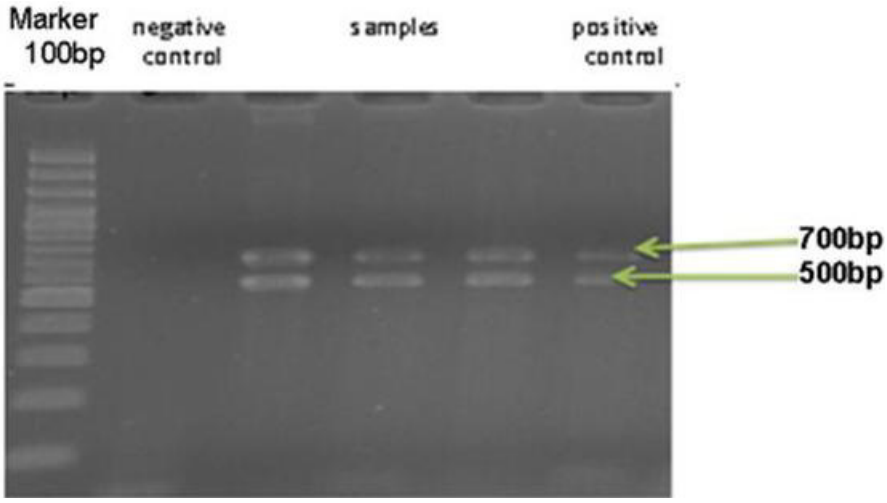

Figure 2. PCR amplification of the *PKS* positive patients. From the left; 100 bp size marker, negative control for clbN and clbB, three positive samples (N4, N9, and N21. The last well in the left shows the PCR result of the positive control.

Table 1. The *PKS* carrier (*K. pneumoniae PKS* positive) individuals and their gender, age, ethnicity, as well as nutritional and habits.

Isolate No.	Age	Kind	Race	Smoking	Use of Meat	Use of Vegetables
N4	60	Female	persian	No	Once a week	yes
N9	38	Female	turkish	No	Third a week	Yes
N21	32	male	persian	No	Once a week	Yes

Table 2. The demographic feature and the antibacterial activity against three *PKS* carrier *K. pneumoniae* isolated from patients as well as bismuth nanoparticles (BINP). Abbreviations are as follow: CIP; ciprofloxacin, NOR; Norofloxacin, Met; metronidazole, and Tet; tetracycline.

Isolate no.	Cip	NOR	Met	Tet	BINP(3500ppm)	BSC $^{250\mu g/ml}$
N_9	40mm (S)	35mm (S)	18mm (S)	15mm (S)	0 (R)	0 (R)
N_{21}	33mm (S)	30mm (S)	15mm (S)	15mm (S)	0 (R)	0 (R)
N_4	42mm (S)	33mm (S)	20mm (S)	14mm (I)	0 (R)	0 (R)

In order to isolate *K. pneumoniae* strains that were *PKS* carrier, PCR was used. A sample of such PCR results is shown in figure 2.

Among the 10 *K. pneumoniae* isolates that were used in PCR analysis, our results showed that 3 isolates were *PKS* positive. These isolates were subjected to further experiments explained below.

Subsequently individuals with CRC (Colorectal Cancer) were identified and their characteristic was summarized as shown in table 1.

Applying disk diffusion test, we investigated the sensitivity of the bacterial isolates to antibiotics and bismuth nanoparticles (BiNp). Our results were compared with those of the Clinical and Laboratory Standards Institute (CLSI) . the pattern of antibiotic resistance was summarized as a table (table 2).

All the *K. pneumoniae* isolates were sensitive to the four antibiotics except the N4 isolate that shows little

Table 3. The pattern of *S. aureus* response to the antibiotics, bismuth nanoparticle (BiNp), as well as bismuth subcitrate (BSN). The bacterium is sensitive to the antibiotics in addition to the applied two types of the bismuth nanoparticles. The antibacterial abbreviations are as follow: CIP; ciprofloxacin, NOR; Norofloxacin, and Tet; tetracycline. R means resistant, I, an intermediate and the S, sensitive accordingly.

Microorgansim	Cip	NOR	Tet	BiNp	BSN
S.aureus	25mm(S)	22mm(S)	20mm(S)	13mm(S)	0(R)

Table 4. The pattern of MBC and MIC test results obtained for the bacterial isolates through application of the antibiotics and the bismuth nanoparticle (BiNp). CIP; ciprofloxacin, NOR; Norofloxacin, Met; metronidazole, and Tet; tetracycline.

Isolate no.	(CIP)		(NOR)		(Tet)		(Met)		BiNp	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
N9	1	2	1	2	2	4	4	8	-	-
N21	0.5	1	2	4	2	4	4	8	-	-
N4	0.5	1	2	4	8	16	2	4	-	-
S.aureus	1	2	3	6	4	8	-	-	$350^{\mu g}/_{ml}$	$350^{\mu g}/_{ml}$

Table 5. The synergistic effect analysis of the antibiotics and bismuth nanoparticles (BiNp) through measurement of MBC and MIC. Abbreviations: CIP; ciprofloxacin, NOR; Norofloxacin, Met; metronidazole, and Tet; tetracycline.

Isolate no.	(CIP)		(NOR)		(Tet)		(Met)		BiNp	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
N9	0.5	1	1	2	2	4	2	4	-	-
N21	0.5	1	2	4	1	2	4	4	-	-
N4	0.25	0.5	2	4	8	16	1	2	-	-
S.aureus	0.5	1	2	4	3	6	-	-	-	-

sensitivity to the tetracycline. As well, all isolates show resistance toward bismuth nanoparticles plus bismuth subcitrate nanoparticles. We further carried out more experiments on the *S. aureus* which not only shows the sensitivity toward all the three types of the antibiotic but also to the bismuth nanoparticles as well (table 3).

Broth tube microdilution and challenge tests

Results obtained for the MBC and MIC tests through application of the antibiotics and bismuth nanoparticles are summarized in the table 4. According to this table in addition to the other results obtained and shown in the previous tables, bacterial isolates show resistant to the bismuth nanoparticles.

To unravel whether there is a synergistic effect between antibiotic and bismuth nanoparticles, further experiment was done the result of which are shown in table 5. A comparison between table 4 and table 5 indicates that there is a synergistic effect between antibiotics and bismuth nanoparticles, but, the extent of synergism is different between antibiotic.

Further analysis was done using challenge test followed by counting bacterial colonies according to the type of the antibiotic and bismuth nanoparticles used in the experiment and concentration. As figure 3 shows a reduction in the bacterial colony formation could be seen which suggests a synergism in the antibacterial activity, but, difference between the kinds of the antibiotics used in the experiment.

DISCUSSION

Multidrug resistance in the pathogenic microorganisms has become a major problem in the present medicine (Falagas ME, Fragoulis KN, 2006). The absence of an appropriate antibiotic to replace the presently used antibiotics for the treatment of the urogenital infections has made the investigation for an effective antibiotic as an inevitable need.

Bismuth is normally found as bismuth sulfide, oxide, and carbonate (Kirk and Othmer, 2004) in clinic and bismuth subsalicylate is used as anti nausea and intestinal problems (Figueroa-Quintanilla D, Salaxar-lindo E, 1993). Nano particles have large surface area. This feature gives them the capability to interact with large biological surfaces.

In the present investigation we studied the antibacterial effect of the bismuth nanoparticles against *Klebsiella pneumoniae* isolates which are carrier of *PKS*. These nanoparticles have not shown an apparent effect as an antibacterial agent. However our results indicate a synergistic effect of the nanoparticles when combined with antibiotics (metronidazole and ciprofloxacin) against

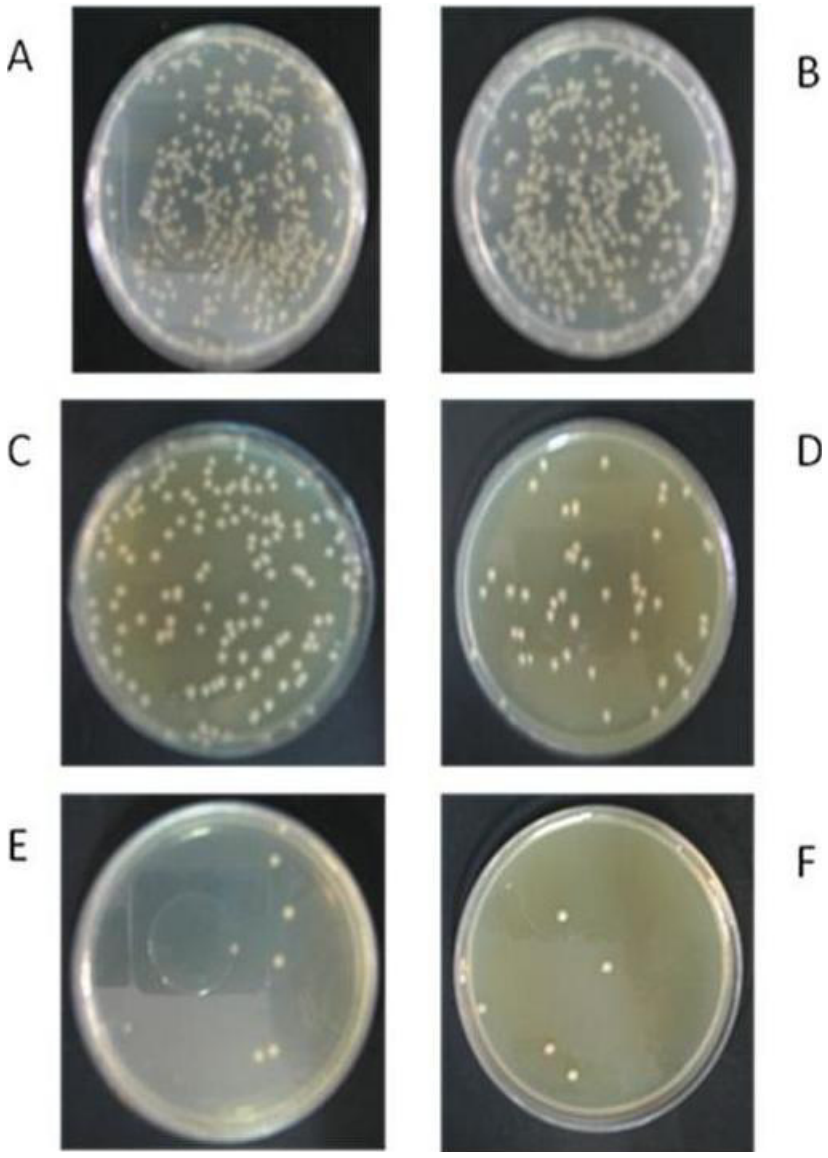

Figure 3. The bacterial growth reduction pattern of the N9 isolate in the presence of the different antibiotics and bismuth nanoparticles. A; control, B; bismuth nanoparticle (3500 ppm), C; sub MIC metronidazole, D; MIC of metronidazole, E; MBC of metronidazole, F; bismuth nanoparticle and MIC of metronidazole.

the three bacterial isolates. Our results show similarity with a study that was carried out by Rajabi and coworkers. In their study they have found antibacterial effect of the bismuth nanoparticles against *E. coli* and *H. pylori*. As well, bismuth subcitrate particles in tetracycline and metronidazole were shown to have synergistic effect on *H. pylori* (Rajabi A, Moazemi N, 1997).

Varopshti and colleagues (2014) have studied the inhibitory effect of bismuth thiols on *Pseudomonas aeroginosa* biofilm. They also have found a synergistic effect for these particles with ciprofloxacin, ceftazidime, and imipenem. Surprisingly we also have found such synergistic effect between bismuth nanoparticles and ciprofloxacin.

The synergism of the bismuth compounds with the antibiotics such as quinolones and ciprofloxacins has also been reported by other investigators (Veloria WG, Domenico P, 2003). In a study carried out by Mohony et al. (1999) they found a higher sensitivity of *H. pylori* and *clostridium* to BSC compared to *E. coli* and *Proteus Mirabilis*. In their study bismuth nanoparticles didn't show antimicrobial effect on *E. coli*. Furthermore Nazari and coworkers have worked on the effect of the bismuth nanoparticles on *H. pylori* and compared it with the other bismuth salts. They found that bismuth salts with a shell of carboxyl group show higher antibacterial activity

compared to bismuth ions. They found a MIC of 60 to 100 µg/ml against *H. pylori* for carboxylated bismuth particles. (Nazari P, 2013).

CONCLUSION

Our study shows that bismuth nanoparticles are not effective if they are applied alone. However, they show synergistic effects when they are used in combination with the other antibiotics. This synergistic effect could be an advantage in using lower amounts of the antibiotics, or, lower combination of antibiotics in the treatment of the infections. Compared to heavy metals bismuth nanoparticles show very low toxic effect. Considering these features, application of the combined antibiotic and bismuth nanoparticles in the treatment of the infections with the *Klebsiella* strains would be an advantage in lowering the antibiotic resistance in this family of microbes. The small size of the nanoparticles combined with their large surface area is a benefit for antimicrobial affects and a mean for illumination of the antibiotic resistance in such strains. Since the studied isolates are *PKS* positive, their elimination is a suitable mean in order to prevent *PKS* transfer to other bacterial strains in the intestine as well as reduction in the possibility of the colorectal cancer.

REFRENCES

Badireddy AR, Rene Hernandez-Delgadillo SC, Sánchez-Nájera RI, Cabral-Romero C (2014). Synthesis and characterization. 116(27):14717-14727.

Briand GG, Burford N (1999). Bismuth compounds and preparations with biological or medicinal relevance. Chem. Rev. 99(9): 2601-58.

Clinical and Laboratory Standards Institute. Mo2, Mo7 tables for *Enterobacteriaceae* (2014) 34(1): 50-58.

Cuevas-Ramos G, Petit CR, Nougayrede JP (2010). *E.coli* induces DNA damage in vivo and triggers genomic instability in mammalian cells. Proc. VaH. Acad. Sci. USA. 107(25): 11537-11542 .

Domenico P (1997). Enhancement of bismuth antibacterial activity with lipophilic thiol chelators. Antimicrob. Agents Chemother. 41(8): 1697-703.

Drummond DC (2006). Development of a Highly Active Nanoliposomal Irinotecan Using a Novel Intraliposomal Stabilization Strategy. Cancer Res. 66(6): 3271-3277.

Falagas ME, Fragoulis KN, Karydis I (2006). A comparative study on the cost of new antibiotics and drugs of other therapeutic categories. PLoS One. 1

Fernandez A, Pereira MJ, Suarez JM, Poza M (2011). Emergenc in resistant Enterobacter Cloacae clinical isolate producing SFO-1 ESBL. J. Clin. Microbiol. 49(3): 822-28.

Figueroa-Quintanilla D, Salaxar-Lindo E, Sack RB (1993). A controlled trial of bismuth subsacylate in infants with acute watery diarrheal disease. New Engl. J. Med. 328(23):1653-

Gangoue PJ, Koulla Sh S, Ngassam P, Adiogo D (2006). Antimicrobial activity against gram negative bacilli from Yaounde Central Hospital, Cameroon. Afr. J. Heaith Sci. 6(4): 232-35.

Hernandez-Delgadillo R (2012). Zerovalent bismuth nanoparticles inhibit Streptococcus mutans growth and formation of biofilm. Int. J. Nanomedicine. 7: 2109-13.

Jean SS, Hsueh PR (2011). High Burden of antimicrobial resistance in Asia. Int. J. Antimicrob. Agents. 37(4): 291-295.

Kirk RE, Othmer DF (2004). Encyclopedia of chemical technology. 5 th ed. Hoboken: John Wiley and sons Inc.

Kopf-Maier P (1994). Complexes of metals other than platinum as antitumour agents. Eur. J. Clin. Pharmacol. 47(1): 1-16.

Leid JG (2012). In vitro antimicrobial studies of silver carbene complexes: activity of free and nanoparticle carbene formulations against clinical isolates of pathogenic bacteria. J. Antimicrob. Chemother. 67(1): 138-48.

Martin HM, Campbell BJ, Hart CA, Mpofu C, Nayar M, Singh R (2004). Enhanced *Escherichia coli* adhberence. Gastroenterology. 127:80-93.

Mohony DE, Lim–Morrison S, Bryden G, Faulkner (1999). Antimicrobial activities of synthetic Bismuth Compounds against *C. difficile*. Antimicrob. Agents Chemoth. 43(3): 582-88.

Nazari P (2013). The Antimicrobial Effects and Metabolomic Footprinting of Carboxyl-Capped Bismuth Nanoparticles Against Helicobacter pylori. Appl. Biochem. Biotechnol. PMID:15236175: http://dx.doi.org/10.1053/j.pastro.2004. 03.054

Padilla E, Liobet E, Pomenech- Sanchez A, Martinez- Martinez L (2010). AcrAB efflux Pump Contributes t, antimicrobial resistance and Virulence. *K.pneumoniae*

Podhorecka M, Skladanowski A, Bozko P, Nougayrede JP, Homburg S, Taieb M, Boury E (2006). *E.coli* induces DNA doubles trand breaks in eukaryotic. Sci. 313:848-51.

Rajabi A, Moazemi N, Mirsalehian A, 1997.effect of bismuth on H. pylori .

Velasco-Arias D (2012). Stabilization of Strong Quantum Confined Colloidal Bismuth Nanoparticles, One-Pot Synthesized at Room Conditions. J.Phy. Chem. C. 116(27)

Veloria WG, Domenico P, Lipuma JJ, Davis JM, Gur zenda E (2003). In vitro activity and synergy of Bismuth thiols and tobramycin against *Bur kholderi cepacia*complex. J. Antimicrob.chemother. 52:915-19.

Wei SW, Q ian W, Ye Y, Ma X (2009). The synthesis of chitosan-based silver Nanoparticles and their antibacterial activity. Carbo hydrate Research. 344: 2375-82.

Willcox RR (1948). The role of bismuth oxychloride in the treatment of syphilis. Practitioner. 161(963): 203.

Yamanaka M, Hara K, Kudo J (005). Bactericidal actions of a silver ion solution on *Escherichia coli*, studied by energy-filtering transmission electron microscopy and proteomic analysis. Appl. Environ. Microbiol. 71(11): 7589-93.