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Sulbactomax prevents antimicrobial resistance development by inhibition of conjugal transfer of F plasmids

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Abstract

Aims: To evaluate the effect of EDTA on conjugation and plasmid transfer also to study the effect of different antibiotics on the conjugation.

Methods and Results: The minimum inhibitory concentration (MIC) of each antibacterial agent was determined using a broth dilution method. *In-vitro* conjugation study was performed in the presence of different concentration of EDTA. Following selection of appropriate concentration of EDTA which inhibits conjugation process and susceptibility profiles, the same concentration was co-related with Sulbactomax and a comparative study against ceftriaxone and ceftriaxone+sulbactam without EDTA was performed. Further to confirm the inhibition of conjugal transfer of plasmid, plasmid DNA was isolated from donor, recipient and transconjugates and processed for electrophoresis. Results of *in vitro* study shows that EDTA when used alone strongly inhibits conjugation process at 10 mM. Sulbactomax at half of MIC strongly inhibited the conjugation process as compared to ceftriaxone and ceftriaxone+sulbactam without EDTA. Further, it is clearly evident from agarose gel electrophoresis that conjugation process is inhibited by EDTA alone at 10 mM and higher as well as Sulbactomax.

Conclusions: The results obtained in the present study, suggests that EDTA alone at 10 mM and at a very low concentration Sulbactomax inhibits the conjugation process and plasmid transfer. Thus, the inhibition of conjugation process is potentially a novel antimicrobial approach in the prevention of transfer of antibiotic resistance.

Significance and Impact of Study: The increasing prevalence of microbial pathogens which are resistant to antibiotics has been encouraging investigation of new strategies for controlling bacterial infections. Conjugative plasmids are potential targets because of the high frequency of antibiotic resistance arising from conjugation and conjugative transfer of plasmid DNA by which antibiotic resistance genes spread between bacterial strains. Since conjugation can happen between closely related (e.g., within Enterobacteriaceae) or distantly related organisms (e.g., grampositive to gram-negative) such as *E coli*. In the present study, EDTA when used alone at 10 mM and at very low concentration in Sulbactomax prevents bacterial conjugation and plasmid transfer effectively and hence prevents development of bacterial resistance ocuuring due to transfer of plasmid DNA.

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Introduction

In the past few decades, antibiotics have been critical in the fighting against infectious disease caused by bacteria and other microbes. Increasing bacterial resistance to an antimicrobial agent is a worldwide problem. The spreading of extended-spectrum beta-lactamases, major cause of antibiotic resistance, is due to mobile genetic elements including conjugative plasmids (Pitout, 2010). Antibiotic sensitivity is variable among bacterial strains due to the presence of plasmids. Plasmids are relatively small, circular DNA molecules that can exist independently of host chromosomes and are found in many bacteria including *E. coli* (Salyers and Gupta, 2004; Jabri, 2008). The genes encoding for resistance to a variety of antibiotics like penicillin and tetracycline are commonly found on plasmids. When a population of susceptible bacteria is exposed to a given antibiotic, most of them will be killed. However, if the population contains cells with conjugative plasmids bearing the genes for resistance, they can rapidly spread the trait throughout the population. Transfer of antibiotic resistance genes need not be restricted to cells of like species. In some cases, this has resulted in disease-causing bacteria that are resistant to almost every antibiotic. Conjugation plasmid transfer via close cell to cell junctions is the main route by which antibiotic resistance genes spread among bacterial strains (Barlow, 2009; Hawkey and Jones, 2009).

Conjugation happened between two mating types: donors and recipients.

For bacteria to achieve conjugation one of the bacteria has to carry the F-plasmid, also known as the F factor, and "partner" bacteria must not have the F-factor. The F-plasmid is a specialized plasmid, known as an episome that is able to integrate itself into the bacterial chromosome and is about 100 kbp in length. The F-plasmid has its own origin of replication (oriV), which is a specific sequence at which DNA replication is originated. Within a single bacterium there can only be a single copy of the F-plasmid, whether that copy is free or integrated into the bacterial chromosome. The F-plasmids encodes for all the proteins necessary for bacterial conjugation, including the proteins necessary to form the pilli, a finger like projection that attaches to the partner bacteria F^+ bacteria possess F factor as a plasmid independent of the bacterial genome. The F plasmid contains only F factor DNA and no DNA from the bacterial genome. F^- bacteria does not contain F factor. When an F^+ cell conjugates/mates with an F^- cell, the result is two F^+ cells, both capable of transmitting the plasmid further by conjugation. After conjugation the recipient cells called transconjugants (Lederberg and Tatum, 1946; Holmes and Jobling, 1996; Ryan and Ray, 2004). Conjugation itself can occur between distantly related species, but some plasmids, such as F plasmid, have a narrow host range (Enterobacteriaceae) due to incompatibilities of the replication system (Guiney, 1982; Frost, 1993).

DNA relaxases are the main enzymes involved in the initiation of conjugative plasmid transfer (Byrd and Matson 1997; Pansegrau and Lanka 1996). Relaxase requires metal ions for cleavage, ligation, and transfer of ssDNA and two catalytic tyrosines, one from each pair (Lujan et al., 2007). The relaxase involved in DNA transfer via a site-and strand-specific ssDNA nick in the transferred strand (T-strand) at the origin of transfer (oriT), forming a covalent 5-phosphotyrosine intermediate (Llosa et al., 2002; Reygers et al., 1991). The nicked T-strand moves from the donor cell (plasmid) to the recipient cell (plasmid) via an intercellular junction (Lujan et al., 2007). Inhibition of relaxases by EDTA would be one of the best choice to prevent conjugative transfer of plasmid. EDTA chelates to metal ions required for the functioning of relaxases and transfer of conjugative plasmid. Thus, inhibition of conjugation by targetting the DNA relaxases enzyme can prolong the use of antibiotics

for antimicrobial therapy as well as it may become useful in the prevention of spreading of antibiotic resistance.

In this study we first tried to develop transconjugants using donor and recipient cells and later identified the concentration of EDTA which inhibits the conjugation between donor and recipient thereafter that information was used to identify the drugs which inhibits bacterial conjugation. Further, susceptibility profiles of donor, recipient and transconjugants were performed against ceftriaxone, ceftriaxone+sulbactam and Sulbactomax. The inhibition of conjugal transfer of plasmid was also confirmed by agarose gel electrophoresis. Our results will establish that the conjugative DNA transfer process can be disrupted by relaxase-targeted compounds, including some clinically approved drugs. This is a novel antimicrobial approach, that can be used in the prevention of antibiotic resistance.

Methods

Bacterial strains

The bacterial conjugation kit provides two *E. coli* strains, A and B referred to as parental strains were procured from Merck Specialities Private limited, Mumbai, India. Strain A carries an antibiotic resistance gene in 'F" plasmid coding for tetracyclin, while strain B is devoid of any 'F" factor but carries streptomycin resistance gene in its chromosome. Therefore both the strains will grow on medium containing the respective antibiotics to which they are resistant. On conjugation the two parental strains will be resistant to both the antibiotics. i.e. the "conjugated sample" when plated on a medium containing both tetracyclin and streptomycin, will survive.

Media

LB broth and agar were supplied with kit. LB broth was prepared by dissolving 12.5 gram of media in 400 ml of distilled water and adjusted the pH 7.0 with 5 N HCl and made the volume to 500 ml with water and sterilized by autoclaving. For LB agar plate, added 1.5% agar into media. The media was provided with respective antibiotics after autoclaving.

Revival of parental strains

The lyophilized vials (donor & recipient *E. coli* strains) were opened and rehydrated each vial with 0.1 ml of sterile LB broth, streaked in duplicates 25 µl of suspension of the donor strain onto LB plates with tetracycline (30 µg/ml) and the recipient strain on LB with streptomycin (100 µg/ml) and innoculated the remaining 50 µl suspension in a tube containing 5 ml LB with respective antibiotics. Incubated these plates and tubes at 37 °C overnight. Inoculated 1 ml of each of donor and recipient into 25 ml LB broth in 250 ml of conical flask with respective antibiotics and incubated at 37 °C.

Preparation of McFarland Standard

McFarland standards are used as turbidity standards in the preparation of suspensions of microorganisms. Briefly, it was prepared by adding 0.5ml of 0.048 M $BaCl_2$ to 99.5 ml of 0.18 M H_2SO_4 with constant stirring. Using matched cuvettes with a 1 cm light path and nutrient broth as a blank standard, measured the optical density in a spectrophotometer at a wavelength of 625 nm. The acceptable range of standard is 0.08 to 0.13.

Antibiotics

Sulbactomax (Ceftriaxone: Sulbactam :: 2:1 with 10 mM EDTA), and ceftriaxone (Rocephin) used in the study were provided by Sponsor Venus Pharma GmbH, Germany and ceftriaxone+sulbactam (Oframax forte) was procured from Indian market on behalf of sponsor for the study. All the antibiotics Sulbactomax, ceftriaxone and ceftriaxone+sulbactam were reconstituted with the water for injection as disclosed in respective packs.

Bacterial conjugation *in vitro*

The strains of *E. coli* (donor) and recipient cells grown in LB broth (10^6 cfu/ml) were mixed at a volume ratio of 1:1 and incubated at 37 °C for 2 hrs. After 2 hrs, added 2 ml of sterile LB broth into each tube and incubated the tubes at 37 °C for another 1.5 hrs. Spread 0.1 ml of each of the samples on plates supplemented with respective antibiotics (donor, recipient, transcojugants). Incubated the plates at 37 °C for 24 hrs and observed the plates.

Effects of EDTA on *in vitro* conjugation

In the F plasmid transfer between strains of *E. coli* (donor) and recipient cells grown in LB broth (10^6 cfu/ml) were mixed at a volume ration of 1:1 and supplemented with different concentrations of EDTA alone, as disclosed below, ranging from 5 mM to 40 mM and incubated at 37 °C for 2 hrs. After 2 hrs, added 2 ml of sterile LB broth into each tube and incubated the tubes at 37 °C for another 1.5 hrs. Spreaded 0.1 ml of each of the samples on plates supplemented with respective antibiotics (donor, recipient, transconjugants). Incubated the plates at 37 °C for 24 hrs and observed.

Concentration of EDTA which is 10 mM is considered as X: 3.7 mg/ml

Concentration X/2 was considered : 5 mM (1.85mg/ml)

Another concentration was 2X : 20 mM (7.5mg/ml)

Still another concentration evaluated was 4X : 40 mM (15mg/ml)

Determination of antimicrobial susceptibility

The minimum inhibitory concentration (MIC) of each antibacterial agent was determined using a broth dilution method with a inoculum size of 10^5 to 10^6 cfu/ml in 96-well microtitre plate. Each well of a 96-well microplate was coated with twofold serial dilutions of Sulbactomax, ceftriaxone and ceftriaxone+sulbactam. The concentration of each drug varies from 64 to 0.03125 µg/ml. A suspension of donor, recipient and transconjugant containing 10^7 to 10^8 cfu/ml, was prepared from a 24 h-old subculture of an agar plate. 0.5 ml of each was put into 9.5 ml LB broth supplemented with respective antibiotic to a density of 10^5 to 10^6 cfu/ml. A 100 µl volume of the suspension was added to each well, and the microtitre plate was incubated at 37 °C for 24 hrs. The MIC was defined as the lowest concentration of a test antibiotic that completely inhibited visible bacterial growth.

Effect of drugs on *in vitro* conjugation

The procedure of mating was the same as that described for the EDTA alone *in vitro* conjugation experiment except 10 mM EDTA and appropriate amount of drug (half of MIC of each drug) was added to conjugation mixture.

Molecular analysis of donor, recipient and transconjugants

The donor, recipient and transconjugants were examined by agarose gel electrophoresis to confirm the transfer of plasmid from donor to recipient and the presence of the same plasmid in transconjugants. Plasmid was isolated using alkaline lysis method as described by Birnboim and Doly (1979) with slight modification. Briefly, donor, recipient and transcojugants were centrifuged at room temperature (25 °C) for 4 minutes at 5000 rpm. Poured off supernatant and drained tube on paper towel. Added 0.2 ml ice-cold solution 1 (25 mM Tris-HCl pH 8.0, 10 mM EDTA pH 8.0 and 50 mM glucose) to cell pellet and resuspended cells using pipette. Then added, 0.4 ml solution 2 (1 % SDS and 0.2 N NaOH) into each tubes and inverted five times gently, allowed to stand all of the tubes at room temperature for 5 minutes. Finally, incorporated 0.3 ml ice-cold solution 3 (3 M Potassium acetate and 5 M glacial acetic acid), into each tubes and inverted five times gently and incubated the tubes on ice for 10 minutes.Centrifuged all of the tubes at 4 °C for 2

minutes at 14000 rpm and transfered supernatant to fresh microcentrifuge tube and added 800 µ.l. of cold Isopropanol and keep for 2 min at room temperature and centrifuge at 14000 rpm for 7 min at 4 °C. Poured off supernatant and washed the pellet with 1 ml of ice-cold 70% ethanol and centrifuged at 4 °C for 7 min at 7500 rpm. Poured off supernatant (be careful not to dump out pellet) and drained tube on paper towel. Added 50 µl of TE to dissolve pellet and processed for electrophoresis.

1.0 % agarose gel was used. 0.5 gram of agarose was taken in a 250 erlenmeyer flask and added 50 ml of 1x electrophoresis buffer (Tris-HCl, 1.0 M, pH 8.0 and EDTA, 0.5 M, pH 8.0) to suspend the agarose powder in the buffer. The gel solution was boiled into the microwave until all of the agarose particles are dissolved. Cooled the molten agarose to 60 °C and added 4 µl of ethidium bromide (10 mg/ml) before pouring on agarose gel slab. After electrophoresis at 70 volt for 15 minutes gel was photographed under UV illumination using gel doc system (Bio-Rad,USA).

Results

***In vitro* conjugation study**

The donor, recipients and transconjugants were gown in the presence of tetracycline (30 µg/ml), streptomycin (100 µg/ml) and combination of both antibiotics, respectively. Results of this experiment revealed that donor was able to grow only with tetracycline and recipient with streptomycin. When donor and recipient were grown on the presence of vice-versa antibiotics, no growth was observed. The transconjugants were able to grow when both of the antibiotics tetracycline and streptomycin were present in the media, (Figure 1), suggesting that gene transfer has taken place from strain A to strain B. However, transconjugants also grow in the presence of individual antibiotic (not shown in figure).

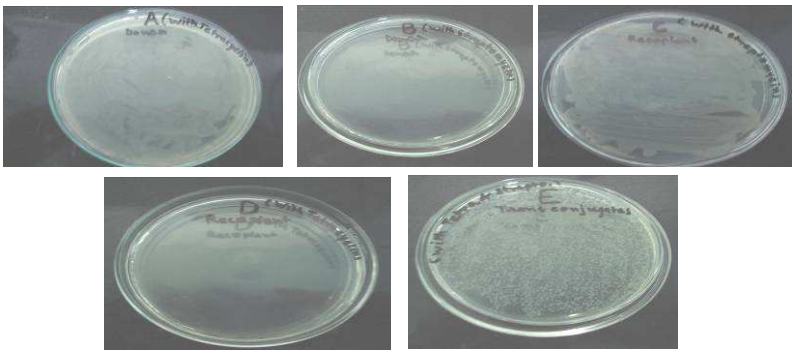

Figure 1: Proof of concept for formation of Transconjugants.
(A) Donor with tetracycline (30 µg/ml); (B)Donor with streptomycin (100 µg/ml)
(C)Recipient with streptomycin (100 µg/ml); (D)Recipient with tetracycline (30 µg/ml)
(E)Transconjugants with tetracycline (30 µg/ml) and streptomycin (100 µg/ml)

Table 1: MIC study of donor, recipient and transconjugants

Name of culture MIC (µg/ml)	Donor	Recipient	Transconjugants
Name of drug			
Ceftriaxone	4	2	4
Ceftriaxone+Sulbactam	1	0.5	1
Sulbactomax	0.25	0.125	0.25

Antimicrobial susceptibility of Sulbactomax, ceftriaxone and ceftriaxone+sulbactam

To assess the potency of Sulbactomax, ceftriaxone and ceftriaxone+sulbactam against donor, recipient and transconjugants, we measured MIC against donor, recipient and transconjugants.The transconjugants exhibited the MIC similar to that of donor strains. However recipient exhibited lesser MIC values.The MIC for Sulbactomax ranged from 0.125 to 0.25 µg/ml and for ceftriaxone and

ceftriaxone+sulbactam, it ranged from 2 to 4 and 0.5 to 1, respectively. Thus, the values of Sulbactomax MIC is several folds less than ceftriaxone and ceftriaxone combined with sulbactam against donor, recipient and transconjugants due to presence of EDTA (Table 1).

Effect of EDTA on *in vitro* conjugation

The effect of EDTA on F plasmid transfer from donor to recipient was investigated and was found that EDTA at 10 mM and higher inhibit the conjugation process. There was no inhibition was observed in conjugation when mating system was provided with EDTA 5 mM (Figure 2).

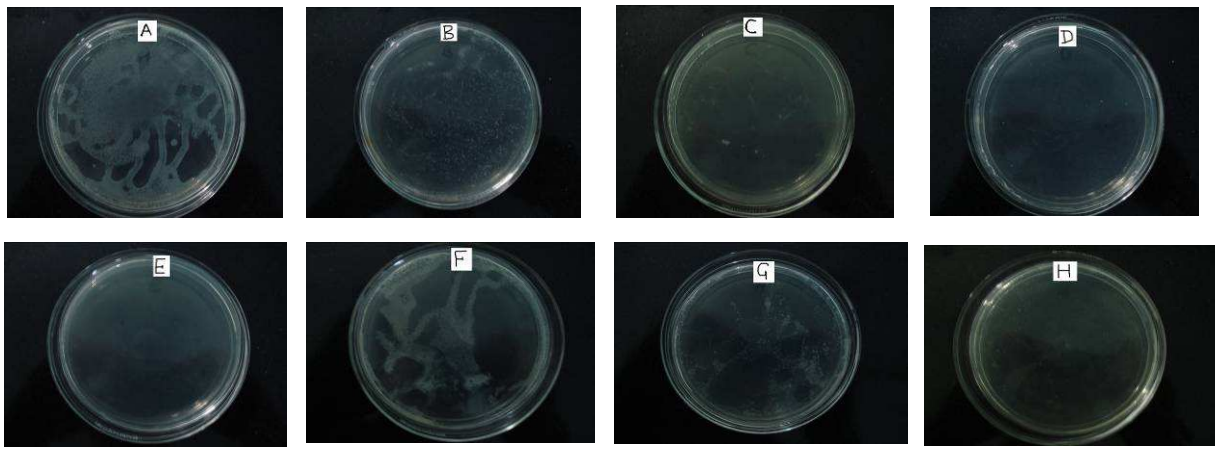

Figure 2: Effect of various concentration of EDTA and Different drugs on conjugation process.
A= Transconjugant (positive control); B = Transconjugant with 5 mM EDTA
C = Transconjugant with 10 mM EDTA; D =Transconjugant with 20 mM EDTA
E = Transconjugant with 40 mM EDTA; F = Transconjugant with ceftriaxone
G = Transconjugant with ceftriaxone+sulbactam; H = Transconjugant with Sulbactomax

Effect of drugs on *in vitro* conjugation

The effect of Sulbactomax, ceftriaxone, ceftriaxone+sulbactam was examined. In the mating of *E coli*. Compound showing low MIC (Sulbactomax 0.25 µg/ml) exhibited inhibition of F plasmid transfer, whereas those showing high MICs (ceftriaxone and ceftriaxone+sulbactam) were less active. (Figure 3).

Molecular analysis of donor, recipient and transconjugants

Agarose gel electrophoretic analysis revealed the absence of resistant plasmid in recipient and presence of resistance plasmid in donor and transconjugants. The transconjugants contained the plasmid of approximately the same size as donor. When the conjugation was carried out in the presence of various concentrations of EDTA, transconjugant formation was inhibited at 10 mM and higher concentrations (Figure 3), resulting in no plasmid bands was observed at 10 mM EDTA concentration and higher. When conjugation was carried out in the presence of various drugs like ceftriaxone, ceftriaxone+sulbactam and Sulbactomax, Ceftriaxone and ceftriaxone+sulbactam treated groups showed the same plasmid size on agarose gel electrophoresis as transconjugants suggesting transfer of resistant plasmid from donor to recipient. On the other hand, Sulbactomax strongly inhibited the formation of transconjugants (Figure 4), indicating only Sulbactomax is able to inhibit the conjugal transfer of bacterial resistant plasmid from donor to recipient as well as killing of bacteria and is not allowing bacterial conjugation to take place, there by preventing resistance development.

a b c d e

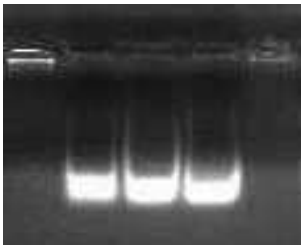

Figure 3: Agarose gel electrophoresis photographs showing EDTA effects on resistant plasmid.

a. *E. coli* recipient (without plasmid)
b. *E. coli* donor (having plasmid with)
c.Transconjugant (TC)
d. TC with 5 mM mg EDTA
e. TC with 10 mM mg EDTA

a b c d e f g

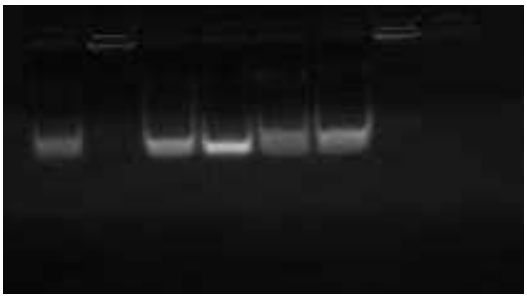

Figure 4: Agarose gel electrophoresis photographs showing effects of different drugs on resistant plasmid.

a: *E. coli* donor (having plasmid)
b: E. coli recipient
c: Transconjugant (TC)
d: TC with 5 mM EDTA
e: TC with Ceftriaxone+ Sulbactam
f: TC with Ceftriaxone
g: TC with Sulbactomax

Discussion

The increasing prevalence of microbial pathogens which are resistant to antibiotics has been encouraging investigation of new strategies for controlling bacterial infections. Conjugative plasmids are potential targets because of the high frequency of antibiotic resistance arising from conjugation and conjugative transfer of plasmid DNA by which antibiotic resistance genes spread between bacterial strains. Since conjugation can happen between closely related (e.g., within Enterobacteriaceae) or distantly related organisms (e.g., gram-positive to gram-negative) such as *E coli*. The spontaneous mutation frequency for antibiotic resistance is on the order of about of about 10^{-8}- 10^{-9}. This means that one in every 10^{8}-10^{9} bacteria in an infection will develop resistance through the process of mutation. In *E. coli*, it has been estimated that streptomycin resistance is acquired at a rate of approximately 10^{-9} when exposed to high concentrations of streptomycin.

In this work, *in vitro* conjugation experiment demonstrates transfer of resistance plasmid from donor to recipient. When conjugation occurs, the plasmid DNA replicates and the newly synthesized copy of the circular F molecule is transferred to the recipient. The recipient cell becomes converted into F^{+} termed as transconjugant. The transfer of the F plasmid from F^{+} to F^{-} is rapid, so the F can spread like wildfire throughout a population from strain to strain. Electrophoretic patterns of donors and transconjugants showed resembling pattern of plasmid in both donors and transconjugants.Plasmid mediated genes, whether they are ESBLs can spread rapidly to members of the same species or organisms of different genera (Pai et al., 2004; Lartigue et al., 2007).

Susceptibility profiles of transconjugants obtained from *in vitro* study were identical to the profile of donor. Sulbactomax having Ceftriaxone: Sulbactam :: 2:1 and 10 mM EDTA has 0.25 µg/ml MIC value and higher bactericidal activity compared to that of ceftriaxone and ceftriaxon+sulbactam. Our results demonstrated EDTA when used alone at 10 mM and higher inhibited the conjugal transfer of F plasmid. Inhibition of conjugation and plasmid transfer does not take place at 5 mM concentration which is X/2, but 10 mM (X) and 2X and 4X effectively inhibited the bacterial conjugation. The inhibition of conjugation by EDTA is due to the inhibition of relaxases at a concentration of 10 mM or higher present alone or in combination with antibitotic Sulbactomax (0.006 µg/ml EDTA at 0.25 µg/ml concentration).The most significant observation of

this study was, when half of MIC level of Sulbactomax was added during conjugation process it also completely inhibited the conjugation process, such inhibition was correlated with the antibacterial activity of the Sulbactomax against transconjugants, suggesting that 10 mM EDTA alone and 0.003 µg/ml in combination with ceftriaxone and sulbactam as present in Sulbactomax at half MIC, have an immediate impact in the prevention of spread of antibiotic resistance and in extending the lifetime of antibiotic. Agarose gel electrophoresis analysis also revealed the inhibition of conjugation process 10 mM EDTA alone and at 0.003 µg/ml in combination with ceftriaxone and sulbactam as present in Sulbactomax. A inhibition in conjugation process was also observed when mating system was provided with Phospholipol (Poole et al., 2006). These results appear to be consistent with previous results that suggested inhibition of the conjugal transfer of F'lac only when the donor is sensitive to the drug (Nakamura et al., 1976).However, there was no relationship between inhibition of F plasmid transfer and bactericidal activity when mating system was provided with ceftriaxone and ceftriaxone+sulbactam. Sulbactomax prevents bacterial conjugation and plasmid transfer effectively and hence prevents development of bacterial resistance ocuuring due to ESBL conjugation.

Conclusions

The experimental results indicate that EDTA when used alone at 10 mM and at 0.003 µg/ml EDTA at half MIC of Sulbactomax inhibites the conjugation process. Thus, the inhibition of conjugative relaxases involved in conjugation process is potentially a novel antimicrobial approach, one that selectively targets bacteria capable of transferring antibiotic resistance and generating multidrug resistant strains.

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References

1) Barlow, M., 2009; What antimicrobial resistance has taught us about horizontal gene transfer. Methods. Mol. Biol. 532, 397-411.
2) Birnboim, H.C., Doly, J. 1979. A Rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Research 7, 1513-1523.
3) Byrd, D.R., Matson, S.W. 1997. Nicking by transesterification: the reaction catalysed by a relaxase. Mol. Microbiol. 25,1011-1022.
4) Frost, L.S., 1993. Conjugative pili and pilus-specific phages. In: Clewell, D.B. (Ed), Bacterial conjugation New York: Plenum Press, pp. 189-212.
5) Guiney, D.G., 1982. Host range of conjugation and replication functions of the *Escherichia coli* sex plasmid Flac. Comparison with the broad host-range plasmid RK2. J. Mol. Biol. 162, 699- 703.
6) Hawkey, P.M., Jones, A.M., 2009. The changing epidemiology of resistance. J. Antimicrob. Chemother. 64, 3–10.
7) Holmes, R.K., Jobling, M.G., 1996. Genetics. In Exchange of Genetic Information. in: Baron S et al, (Eds.) Baron's Medical Microbiology, 4th ed., Univ of Texas Medical Branch.
8) Jabri, R.R.G., 2008. Role of conjugative plasmids in antibiotic resistance between two strains of *E. coli*. Eng. & Technol. 26, 1429-1437.
9) Lartigue, M.F., Zinsius, C., Wenger, A., Bille, J., Poirel, L., Nordmann, P., 2007. Extended-spectrum β- lactamases of the CTX-M type now in Switzerland. Antimicrob. Agents.Chemother. 51, 2855- 2860.
10) Lederberg, J., Tatum, E.L., 1946. Gene Recombination in *E. coli*. Nature 158, 558.
11) Llosa, M., Gomis-Ruth, F.X., Coll, M., de la Cruz, F.d. F., 2002. Bacterial conjugation: a two-step mechanism for DNA transport. Mol. Microbiol. 2002; 45, 1–8.

12) Lujan, S.A., Guogas, L.M., Ragonese, H., Matson, S.W., Redinb, M.R.,2007. Disrupting antibiotic resistance propagation by inhibiting the conjugative DNA relaxase. Proc. Nat. Acad. Sci. 104, 12282-12287.

13) Nakamura, S., Inoue, S., Shimizu, M., Iyobe, S., Mitsuhashi, S., Inhibition of conjugal transfer of R plasmids by pipemidic acid and related compounds. Antimicrob. Agents Chemother. 10, 779- 785.

14) Pansegrau, W., Lanka, E., 1996. Enzymology of DNA transfer by conjugative mechanisms. Prog. Nucleic Acid Res. 1996; 54, 197-251.

15) Pai, H., Kang, C.I., Byeon, J.H., Lee, K.D., Park, W.B., Kim, H.B. et al. 2004. Epidemiology and clinical features of bloodstream infections caused by AmpC-type-β-lactamase-producing *Klebsiella pneumoniae*. Antimicrob. Agents. Chemother. 48, 3720-3728.

16) Pitout, J.D., 2010. Infections with extended-spectrum beta – lactamase - producing enterobacteriaceae: changing epidemiology and drug treatment choices. Drugs 70, 313-333.

17) Poole, T.L., McReynolds, J.L., Edrington, T.S,. Byrd, J.A., Callaway, T.R., Nisbet, D.J., 2006. Effect of flavophospholipol on conjugation frequency between *Escherichia coli* donor and recipioent pairs *in vitro* and in the chicken gastrointestinal tract. J. Antimicro. Chemother. 2006; 58, 359-366.

18) Reygers, U., Wessel, R., Muller, H., Hoffmann-Berling, H., 1991. Endonuclease activity of *Escherichia coli* DNA helicase I directed against the transfer origin of the F factor. EMBO J. 10, 2689-2694.

19) Ryan, K.J., Ray, C.G., 2004. Sherris Medical Microbiology. 4th ed., McGraw Hill, pp. 60-64.

20) Salyers, A.A., Gupta, A., Wang, Y., 2004. Human Intestinal Bacteria as Reservoirs for Antibiotic Resistance Genes. Trends Microbiol. 12, 412-6.