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Anti - Microbial Plasma Membrane Activity of Daptomycin and Pantoyl Lactone against *Streptococcus equi*

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Abstract- In this study, the ability of two antibiotics daptomycin and pantoyl lactone to inhibit growth of horse originated pathogen Streptococcus equi by disrupting plasma membrane of the bacterium was investigated using agar diffusion and micro tube dilution methods. The results obtained from this study indicated that daptomycin was more effective in inhibiting the growth of the pathogen in vitro than the second antibiotics. The 3 tested strains were susceptible to daptomycin but completely resistant to pantoyl lactone. Hence, daptomycin therefore tends to be potent for treating horse infections caused by this pathogen.

Index Terms- plasma membrane, agar diffusion, antimicrobial

I. INTRODUCTION

The membrane of *Streptococcus equi*, like other micro-organisms contains lipids and proteins which are the major components of all membranes (Kakizaki et al., 2002). Below is an overview of some of the major components of the *S. equi* membrane. Gram - positive bacteria such as *S. equi* have developed mechanisms for anchoring of proteins to their membranes via covalent N-terminal lipidation (Sutcliffe & Russell, 1995). The lipidation process occurs during the export to the cell surface. Lpp form significant proportion of bacterial membrane; hence they may participate in a wide variety of functions including metabolic regulation, nutrient acquisition and signal transduction. They may also contribute to the virulence of some bacterial pathogens (Harrington et al., 2002). Lactone compounds are widely distributed in the nature and play important roles in microorganisms. Lactone-hydrolyzing enzymes may be involved in the synthesis and degradation of such lactone compounds (Rose, 1976). Pantoyl lactone is an anti-microbial compound that has been found to bring about changes in the cell membrane lipids of microorganisms such as *Micrococcus lysodeikticus* (Johnson et al., 1980). Significant amounts of the two major phospholipids (phosphatidylglycerol and diphosphatidylglycerol) were converted to lyso-forms, which are compounds resulting from the hydrolysis of one of the fatty acids (usually the β) present in phosphodiacylglycerol (Devlin, 1997). Pantoyl lactone has also been shown to inhibit the uptake of glycerol, pyruvate, malate, 2-deoxy-D-glucose, uracil and several amino acids (Grula and King, 1971). Johnson et al., (1980) reported that the hydroxyl and carbonyl functional groups of Pantoyl lactone interact with lipids of the cell membrane, most likely through hydrogen bonding, whereas the methyl group is hydrophobic. This interaction led to the damage of cell membrane, as revealed by scanning calorimetry, thereby inhibiting cell division (Johnson et al., 1980). Hence this compound has been shown to affect bacterial membranes. However, its effects on *S. equi* have never been reported. It is one of the aims of this project to investigate the effect this anti-microbial agent will have on the membrane lipids of *S. equi*. Daptomycin is a cyclic lipopeptide, which is used as anti-bactericidal agent against a wide range of gram-positive bacterial pathogens, including the antibiotic resistant preumococci, enterococci, and staphylococci that are currently presenting a challenge for the development of empirical chemotherapy (Barry et al., 2001). Tally et al., (1999) reported that daptomycin is currently being evaluated for possible applications in cases in which there may be a high prevalence of antibiotic-resistant gram-positive bacteria such as *Streptococci* and *Staphylococci*. The bacterial activity of daptomycin requires the presence of calcium cations. Jones et al., (1987) recommend that when testing for daptomycin, the broth media should contain additional calcium approaching the concentration of ionized calcium that is normally found in human serum (e.g. 50mg/l). Barry et al., (2001) reported that *Staphylococci* and *Streptococci* were inhibited by daptomycin at 2μg/ml when tested in calcium supplemented with Cation-adjusted Mueller-Hinton broth (CAMHB). In recent years, attention has been focused on minimum inhibitory concentrations of daptomycin ranging from 2.0 to 4.0 μg/ml have been determined when assessing the effect of additional calcium to the broth medium (Barry et. al., 2001). Thus, several studies indicated that daptomycin might be useful for treating serious enterococcal infections (Kennedy & Chambers, 1989; Ramas et al., 1992). Despite the potent antibacterial ability of daptomycin, its mechanism of action has not been clearly understood. However, two different mechanisms of action have been proposed for its bacterial activity, involving either the inhibition of LTA synthesis (Boaretti & Canepari, 1995) or the dissipation of the membrane potential across the cytoplasmic membrane, leading to the disruption of several different cellular processes (Alborn et al., 1991). Recently, Silverman et al. (2003) proposed a multi-step model for the mechanism of action of daptomycin that involves the depolarisation of the cytoplasmic membrane. Jung et al., (2004) have also attempted to elucidate the mechanism of action of daptomycin by significantly modifying the model of Silverman et al., (2003) by demonstrating that Ca^{2+} is required for two distinct

conformational changes, each of which separately impacted how daptomycin interacted with membranes.

In this study we are aiming to bring to an end the suffering of horses by identifying antibiotics that might inhibit growth of *S. equi* by focusing on selected compounds (chemicals/antibiotics) that may interact with *S. equi* membrane component resulting in the rupture and killing of the bacterium or inhibition of its growth. The compounds investigated in this study are chosen for their likely ability to interact, dissolve into and affect membranes.

II. MATERIALS AND METHODS

Source of bacteria

The following three strains of *S. equi* 4047, K3 and NCTC 9682 strains were used in this study and obtained from Dr. I. Sutcliffe, Northumbria University, United Kingdom). The strains were firstly cultured in Brain Heart Infusion (BHI) broth overnight and incubated at 37 ^{o} C. Incubation was until the broth became turbid. Biochemical tests and Gram staining were also used to ascertain that the bacteria are gram-positive cocci in chains. Pure culture of each bacterial strain obtained by aseptically streaking a loopful inoculum on to solidified (BHI agar). The plates were incubated for 18 – 24 hours at 37 ^{o}C overnight. Stock culture were then prepared from the pure cultures and kept at 4 ^{o}C in the refrigerator for further study.

Media preparation

Brain Heart Infusion (BHI) broth

This was prepared according to manufacturer's instruction. Briefly, 7.4 g of granulated BHI was weighed before adding it to 200 ml of distilled water. This was mixed thoroughly for the solute to dissolve before distributing 20 ml into 10 universal bottles. All the bottles were autoclaved at 121 ^{o} C for 15 minutes.

Preparation of calcium supplemented BHI agar

This was prepared as described by Zidan (2012).

Preparation of antimicrobial compounds:

Preparation of pantoyl lactone (PL) stocks.

Two methods for preparing PL stocks were carried out. In first method, 0.36 mg of pantoyl lactone (PL) powder was weighed into sterile tubes before adding 200 ml of distilled water. The tubes were shaken thoroughly so as to enable the solute to dissolve. Prepared stock was then refrigerated for further use. However, the second method was as follows: 200 mg/ml stock of PL was prepared by weighing out 600 mg of PL and dissolving this into 3 ml of sterilized BHI broth which was well mixed in a bijou tube and filtered by use of membrane filter (for sterilization) and kept in the freezer as a stock.

Preparation of 20 mg/ml daptomycin stock.

This was prepared by weighing 36mg of daptomycin into a sterile eppendorf and dissolving in 20mg/ml of sterile distilled water. The prepared stock solution was clearly labelled and stored in the freezer.

Broth (tube) assay for MICs of antibiotics.

Starter culture was prepared by inoculating two McCartney tubes containing 20ml of BHI broth by a single colony of *Strep. equi* 4047 and then kept in the incubator at 37^{o}C for 24 hours. Duplicate inocula consisting of 20 ml broth were necessary to avoid failure of growth in one of the McCartney tubes. The tubes and plates were then placed in a closed jar containing a lit candle to create an anaerobic condition then incubated at 37^{o}C overnight. The jar containing the tubes and plates was taken out of the incubator to be examined. The tubes were arranged in an ascending order in a rack (i.e. tube 1 to tube 12) for observation. The tubes with bacteria growth were scored as positive (+) while the tubes without bacteria growth were scored as negative (-). Scoring the results starts from the control tube, which was 12th in which bacterial growth is expected because no antibiotic was added into it. Next is the scoring of 11th tube with the lowest antibiotic concentration, and so on. MIC is taken as the lowest concentration at which no growth. The agar plates were examined by counting the number of colonies, particularly on the plate marked with 10^{-4} dilution. The result was used to calculate the viable counts (c.f.u/ml) in the starting inocula.

Broth assay for other antibiotics

In the case of the revised method for pantoyl lactone from the stock previously prepared (the second method) as mentioned earlier, 1ml was transferred into the first test-tube that contained 1ml BHI broth and labelled as tube 1 with 100 mg/ml concentration of pantoyl lactone. Then from tube 1, two fold serial dilutions were carried out up to tube 11 from which 1ml was discarded. Tube 12 was kept as a control. The final concentrations of pantoyl lactone in the various tubes were recorded.

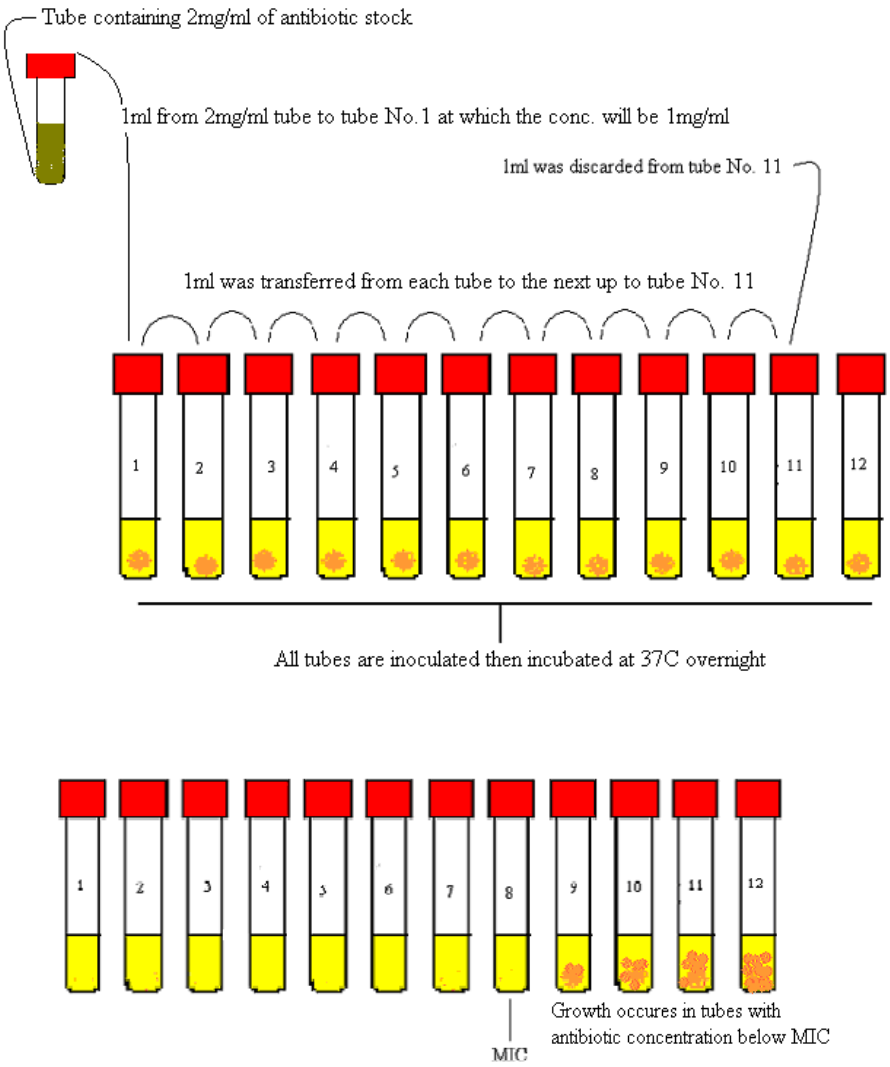

Figure1: Shows MIC determination by two-fold serial dilution

Table 1: Shows the final concentrations of pantoyl lactone in the various tubes.

Tube number	Pantoyl lactone addition (mL)	Broth addition (mL)	Final pantoyl lactone concentration (mg/mL)
1	1 (from 200 mg/l stock)	1	100
2	1 (from tube 1)	1	50
3	1 (from tube 2)	1	25
4	1 (from tube 3)	1	12.5
5	1(from tube 4)	1	6.3
6	1(from tube 5)	1	3.1
7	1(from tube 6)	1	1.6
8	1(from tube 7)	1	0.8
9	1(from tube 8)	1	0.4
10	1from tube 9)	1	0.2
11**	1(from tube 10)	1	0.1
12	0	1	0

In the case of daptomycin, to prepare 40 mg/L, by transferring 8µl of the stock into 4ml BHI+Ca broth in a sterile tube. Two fold serial dilutions were carried out up to tube 11, from which 1ml was discarded. Tube 12 was kept as a control. The final concentrations of daptomycin in the various tubes also recorded.

Table 2: Shows the final concentrations of daptomycin in the various tubes.

Tube number	Daptomycin addition (mL)	BHI+Ca Broth addition (m/L)	Final antibiotic concentration (mg/L)
1	1 (from 40mg/L (stock)	1	20
2	1 (from tube 1)	1	10
3	1 (from tube 2)	1	5
4	1 (from tube 3)	1	2.5
5	1(from tube 4)	1	1.25
6	1(from tube 5)	1	0.625
7	1(from tube 6)	1	0.312
8	1(from tube 7)	1	0.156
9	1(from tube 8)	1	0.078
0	1from tube 9)	1	0.039
11**	1(from tube 10)	1	0.020
12	0	1	0

Plate assay

Disc diffusion assay of daptomycin.

Both starter culture and inoculum for each strain was prepared as earlier described Each strain was inoculated onto calcium supplemented BHI plate by adding 25µl from the inoculum onto each plate, which was spread using a sterile glass spreader while sterile forceps were used to place daptomycin sensitivity disc into each of the inoculated plates. All plates were then incubated under anaerobic conditions for 24 hours at 37^{o}C.

III. Results & Discussion

Pantoyl lactone

Various concentrations of pantoyl lactone were tested for inhibitory ability by observing visible growth shown as turbid in all the tubes (Table 1). Results obtained indicated that the MIC was > 1 mg/ml. Thus the concentration of pantoyl lactone was revised; the concentration range was increased to 100 mg/ml in subsequent experiments as shown in Table 3. Using these revised concentrations, there was no growth in tubes 1-4 in the first two experiments, while growth occurred in tubes 5-12; this means the MIC in this case was ≥ 12.5 mg/ml. Four more experiments were conducted (Table 3) to confirm the MIC ≥ 12.5 mg/ml obtained in the first two experiments. However, growth was observed in all the tubes, including tube No.1, with a concentration of 100 mg/ml. Therefore, the MIC ≥ 100 mg/ml indicates that *S. equi* 4047 is resistant to pantoyl lactone with this concentrations range 0.1mg/ml-100mg/ml. Pantoyl lactone assay with other strains. Pantoyl lactone assay was performed on the three strains of Strep. equi 4047, K3 and NCTC 9682 within the concentration range of 0.1mg/ml-100mg/ml. Growth was observed in all the tubes of the three strains as illustrated in Table 4, thus confirming that the three strains are resistant to pantoyl lactone within this concentration range. In contrast, media containing a

concentration of 0.15M (0.0195g/ml) pantoyl lactone has been reported to significantly inhibit growth and transport activities in Micrococcus lysodeikticus (Johnson et al., 1980). This inhibition was brought about by the modification of membrane phospholipids to lyso forms. Despite the alteration of the cell membrane composition of *Micrococcus lysodeikticus* when grown in pantoyl lactone, normal cell growth and transport activities resume when the cells are washed free of pantoyl lactone (Johnson et al., 1980).

Table 3. Shows action of Pantoyl Lactone for *S. equi* strain 4047 (Revised method)

Expt NO.	Inoc c.f.u/ml	Tube number, Antibiotic final concentration & Growth state +/-											
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	1 x107	1mg /mL	500 g/ml	250g/ ml	125 g/ml	62.5 g/ ml	31 g/m l	15.6 g/ ml	7.8 g/ ml	3.9 g/ ml	2.0 g/ ml	1.0 g/ml	Zero
2	4.2 x10 ^{6}	100 mg/ mL	50m g/ml	25mg /ml	12.5 mg/ ml	6.3mg/ ml	3.1mg/ ml	1.6mg/ ml	0.8mg/ ml	0.4mg/ ml	0.2mg/ ml	0.1m g/ml	zero
		_	_	_	_	+	+	+	+	+	+	+	+
3	5.5 x10 ^{6}	100 mg/ mL	50m g/l	25mg /l	12.5 mg/l	6.3mg/ ml	3.1mg/ ml	1.6mg/ ml	0.8mg/ ml	0.4mg/ ml	0.2mg/ ml	0.1m g/ml	zero
		_	_	_	_	+	+	+	+	+	+	+	+
4	4. 5 x10 ^{6}	100 mg/ L	50m g/l	25mg /l	12.5 mg/l	6.3mg/ ml	3.1mg/ ml	1.6mg/ ml	0.8mg/ ml	0.4mg/ ml	0.2mg/ ml	0.1m g/ml	zero
		+	+	+	+	+	+	+	+	+	+	+	+
5	2.5 x 10 ^{6}	100 mg/ L	50m g/l	25mg /l	12.5 mg/l	6.3mg/ ml	3.1mg/ ml	1.6mg/ ml	0.8mg/ ml	0.4mg/ ml	0.2mg/ ml	0.1m g/ml	zero
		+	+	+	+	+	+	+	+	+	+	+	+
6	7.5 x 10 ^{6}	100 mg/ L	50m g/l	25mg /l	12.5 mg/l	6.3mg/ ml	3.1mg/ ml	1.6mg/ ml	0.8mg/ ml	0.4mg/ ml	0.2mg/ ml	0.1m g/ml	zero
		+	+	+	+	+	+	+	+	+	+	+	+
7	3.4 x 10 ^{6}	100 mg/ L	50m g/l	25mg /l	12.5 mg/l	6.3mg/ ml	3.1mg/ ml	1.6mg/ ml	0.8mg/ ml	0.4mg/ ml	0.2mg/ ml	0.1m g/ml	zero
		+	+	+	+	+	+	+	+	+	+	+	+

Table 4. Shows action of Pantoyl Lactone for *Strep. equi* strains 4047, K3, NCTC 9682.

Tube NO.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Pantoyl lactone concentration	**100m g/mL**	**50mg /ml**	**25mg /ml**	**12.5 mg/m l**	**6.3m g/ml**	**3.1m g/ml**	**1.6m g/ml**	**0.8m g/ml**	**0.4m g/ml**	**0.2m g/ml**	**0.1m g/ml**	**0**
S. equi 4047 Growth	+	+	+	+	+	+	+	+	+	+	+	+
S. equi K3 Growth	+	+	+	+	+	+	+	+	+	+	+	+
S. equi NCTL 9682 Growth	+	+	+	+	+	+	+	+	+	+	+	+

Daptomycin assay.

The daptomycin results are shown in Table 5, which indicates that the MICs of daptomycin for the three strains is ≥ 1.25µg/ml.

Table 5. Shows action of Daptomycin for *Strep. equi* strains 4047, K3 and NCTC 9682.

Tube NO.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Daptomycin concentration	**20 mg/L**	**10µg/l**	**5µg/l**	**2.5 µg/l**	**1.25µg/l**	**0.625µg/l**	**0.312µg/l**	**0.156µg/l**	**0.078 µg/l**	**0.039 µg/l**	**0.20µg/l**	**0**
S. equi 4047[1] Growth	-	-	-	-	-	+	+	+	+	+	+	+
S. equi K3[2] Growth	-	-	-	-	-	+	+	+	+	+	+	+
S. equi NCTL 9682[3] Growth	-	-	-	-	-	+	+	+	+	+	+	+

1- Strain 4047 3.7×10^5 c.f.u /ml
2- Strain K3 4.35×10^5 c.f.u /ml
3- Strain NCTC 9682 3.10×10^6 c.f.u /ml

The experiment was repeated twice as shown in Tables 19 and 20, in order to confirm the MICs for the different strains. The MIC of daptomycin on strain 9682 was confirmed to be ≥ 1.25µg/ml. However, there were slight variation of the MICs on the other two strains which was confirmed to be ≥ 0.625µg/ml (see Tables 18 and 19).

Table 6. Shows action of Daptomycin for *Strep. equi* strains 4047, K3 and NCTC 9682.

Tube NO.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Daptomycin concentration	**20 mg/L**	**10µg/l**	**5µg/l**	**2.5 µg/l**	**1.25µg/l**	**0.625µg/l**	**0.312µg/l**	**0.156µg/l**	**0.078 µg/l**	**0.039 µg/l**	**0.20µg/l**	**0**
S. equi 4047[1] Growth	-	-	-	-	-	-	+	+	+	+	+	+
S. equi K3[2] Growth	-	-	-	-	-	-	+	+	+	+	+	+
S. equi NCTL 9682[3] Growth	-	-	-	-	-	+	+	+	+	+	+	+

1- Strain 4047 5.2×10^5 c.f.u /ml
2- Strain K3 3.28×10^5 c.f.u /ml
3- Strain NCTC 9682 2.12×10^6 c.f.u /ml

Table 7. Shows action of Daptomycin for *Strep. equi* strains 4047, K3 and NCTC 9682.

Tube NO.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Daptomycin concentration	**20 mg/L**	**10µg/l**	**5µg/l**	**2.5 µg/l**	**1.25µg/l**	**0.625µg/l**	**0.312µg/l**	**0.156µg/l**	**0.078 µg/l**	**0.039µg/l**	**0.20µg/l**	**0**
S. equi 4047[1] Growth	-	-	-	-	-	-	+	+	+	+	+	+
S. equi K3[2] Growth	-	-	-	-	-	-	+	+	+	+	+	+
S. equi NCTL 9682[3] Growth	-	-	-	-	-	+	+	+	+	+	+	+

1- Strain 4047 6.7×10^5 c.f.u /ml
2- Strain K3 9.8×10^5 c.f.u /ml
3- Strain NCTC 9682 2.10×10^6 c.f.u /ml

Daptomycin disc diffusion assay

Four experiments were conducted for daptomycin disc diffusion assay for the three strains of *S. equi*, and the results were as shown in Figure 7. Discs led to the appearance of a clear zone for all the strains. The diameters of the clear zones were measured and presented in Table 8.

Table 8. Shows diameters of the clear zones of daptomycin disk.

***Strep. equi* strains**	**diameters in mm for different experiments**				**mean ± sd**
4047	27	31	26	28	28 ± 1.87
K3	22	25	22	23	23 ± 1.22
9682	25	29	25	26	26.2 ± 1.64

The mean diameter of each of the strains was > 16. Therefore, according to daptomycin Kirby-Bauer interpretive criteria all the three strains of *Strep. equi* are sensitive to daptomycin.

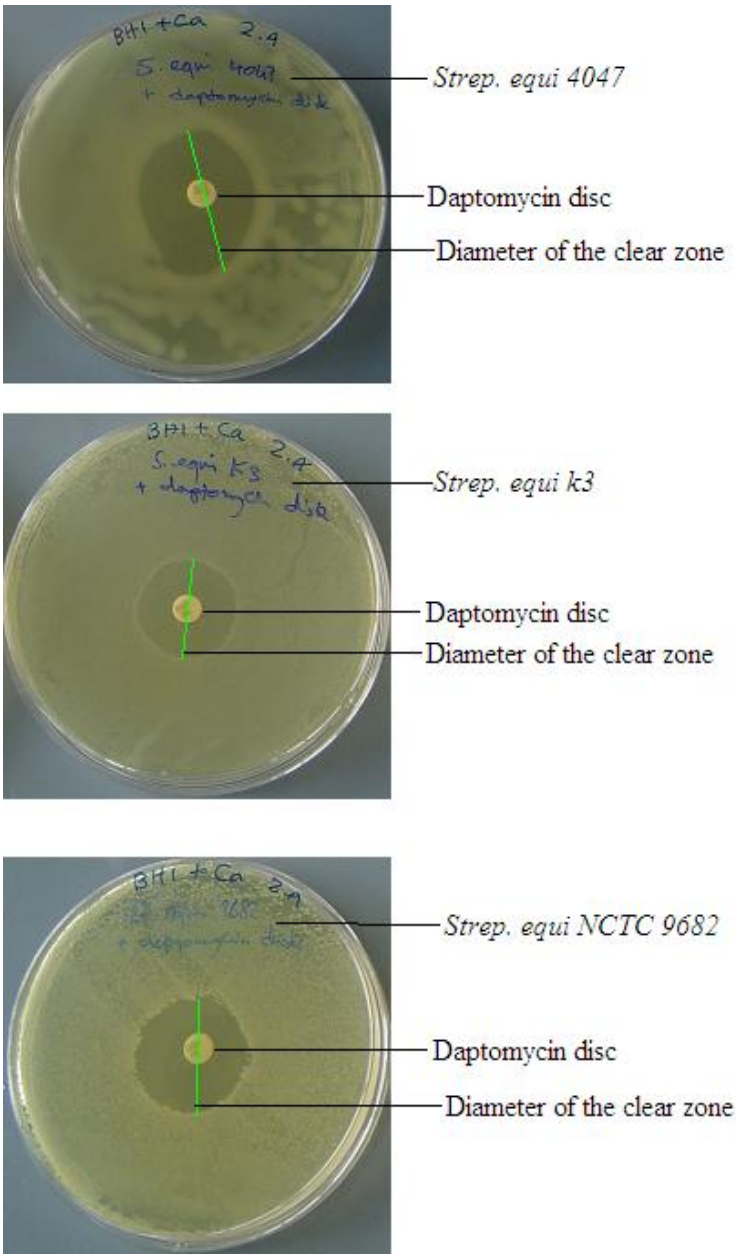

Fig 2. Daptomycin disk assay for Strep. equi 4047, K3 and NCTC 9682.

IV. Conclusion

Daptomycin has been reported to have a potent antibacterial action on a wide range of micro-organisms including against vancomycin-susceptible and vancomycin-resistant enterococci (Barry et al., 2001). The highly sensitive response of the three strains of *S. equi* obtained in this work is in agreement with that represented by Barry et al., (2001), in which almost all *Staphylococci and Streptococci* strains were inhibited at 2 mg/ml when tested in calcium-supplemented. Hence, daptomycin therefore tends to be potent for treating horse infections caused by this pathogen

References

[1] Alborn, W.E., Allen, N.E., and Preston, D.A.(1991). Daptomycin disrupts membrane potential in growing *Staphylococcus aureus.* Antimicrob. Agents Chemother. **35**, 2282-2287.

[2] Barry, A. L., P. C. Fuchs, and S. D. Brown**.** 2001. In vitro activities of daptomycin against 2,789 clinical isolates from 11 North American medical centers. Antimicrob. Agents Chemother. **45:**1919-1922.

[3] Boaretti, M., and Canepari, P.(1995). Identification of daptomycin-binding proteins in the membrane of *Enterococcus hirae.* Antimicrob. Agents Chemother. **39,** 2068-2072.

[4] Devlin T, M. 1997. Textbook of biochemistry with clinical correlations. Fourth edition, wiley-liss,.Inc. New York.

[5] Grula, E.A., and King, R.D. (1971). Changes in cell membrane of dividing and nondividing cells of *Micrococcus lysodeikticus* disIIp- Biochem. Biophys, Res. Commun. **44**,1356.

[6] Harrington D. J., Sutcliffe I. C., Chanter N. (2002). The molecular basis of *Streptococcus equi* infection and disease. Microbes and Infection, **4**, 501-510.

[7] Kakizaki I, Keiichi Takagaki, Yasufumi Endo, Daisyke Kydo, Hitoshi Ikeya, Taruzo Miuoshi, Bruce A. Baggenstoss, Valarie L. Tlapak-Simmons, Kshama Kumari, Akio Nakane, Paul H. Weigel and Masahiko Endo. (2002). Inhibition of hyaluronan synthesis in *Streptococcus equi* FM100 by 4-methylumbelliferone. Eur. J. Biochem. **269,** 5066-5075.

[8] Johnson J.H., Grula E.A, Richard Staerkel, Fung B.M, and Mangum B.L.(1980) Modification of cell membrane lipids in *Micrococcus lysodeikticus* induced by Pantoyl lactone. J. Membrane Biol. **56**, 49-53.

[9] Jones R. N., and Barry A. L (1987). Antimicrobial activity and spectrum of LY 146032, a lipopeptide antibiotic, including susceptibility test recommendations. Antimicrob. Agents Chemother. **31:** 625-629.

[10] Kennedy S and Chambers H. F (1989). Daptomycin (LY146032) for prevention and treatment of experimental aortic valve endocarditis in rabbits. Antimicrob. Agents Chemother. **33:** 1522-1525.

[11] Ramas G. M., Grayson M. L., Eliopoulos G. M and Bayer A. S (1992). Comparison of daptomycin, vancomycin, and ampicillin-gentamicin for treatment of experimental endocarditis caused by penicillin-resistant enterococci. *Antimicrob. Agents Chemother*. **36:** 1864-1869.

[12] Rose A. H., (1976). Chemical Microbiology an introduction to microbial physiology. Third edition,. Butterworth & co. Ltd. Norwich.

[13] Silverman, J.A., Perlmutter, N.G, and Shapiro, H.M, (2003). Correlation of daptomycin bactericidal activity and membrane depolarization in *Staphylococcus aureus.* Antimicrobial Agents And Chemotherapy. **47**, pp. 2538-2544

[14] Sutcliffe I. C & Russell R. R. B (1995). Lipoproteins of Gram-positive bacteria. J bacterial **175**, 1123-1128.

[15] Tally, F.P., Zeckel, M., Waselewski, M.M., Carini, C., Berman, C.L., Drusano, G.L., and Oleson, F.B (1999). Daptomycin: a novel agent for Gram-positive infections. Exp. Opin. Investig. Drugs **8**: 1223-1238.

[16] Zidan A. Bashir. In Vitro Antimicrobial Activity of Membrane - Acting Antibiotics Action Against *Streptococci*. J App Pharm Sci. 2012; 2 (12): 042-047.

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