Antimicrobial activities of lactic acid bacteria against *Pseudomonas aeruginosa, Providencia vermicola, Alcaligenes faecalis* and methicillin resistant *S. aureus*

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ABSTRACT

Background: There is rapid emergence of drug-resistant methicillin resistant *S. aureus* (MRSA) pseudomonads and enterobacteriaceae strains, however little information are available on effects of lactic acid bacteria (LAB) on some enterobacteriaceae strains.

Objective: This study was to assess the antimicrobial effect of some lactobacilli and *Weisella* strains against selected potential pathogens (*Pseudomonas aeruginosa,* MRSA*, Providencia vermicola* and *Alcaligenes faecalis)* in co-culture.

Methods: The antibiotic susceptibility of the selected potential pathogens was done by disc diffusion method and cell free supernatant of fourteen different strains of LAB was initially tested against the selected potential pathogens by agar diffusion method..The antimicrobial effects of *Lactobacillus plantarum* 9, *Lactobacillus buchneri* SM04, *Lactobacillus fermentum* 008, *Lactobacillus brevis* 21, and *Weissella paramesenteroides* BS03 were tested against the four selected potential pathogens by two different co-culture methods at different contact times.

Results: The tested potential pathogens were generally resistant to tested antibiotics with *Providencia. vermicola* exhibiting 100% resistance. *Lactobacillus brevis* 21*, Lactobacillus buchneri* SM04*, Lactobacillus plantarum* 9*, Lactobacillus fermentum* 008, and *Weissella paramesenteroides* BS03 have antimicrobial activities against the tested potential pathogens in cell free supernatant experiment. The five tested LAB inhibited the potential pathogens in co-culture but at different contact time. *Lactobacillus fermentum* 008 totally inhibited the growth of *Providencia vermicola* after 18 hours of co-culture while all the potential pathogens were inhibited in overnight culture of LAB.

Conclusion: Lactic acid bacteria have great antagonistic activities against the tested potential pathogens used in this study. They therefore have great potentials as alternative therapy in cases where antibiotics resistance has been established.

Keywords: Lactobacillus, resistance, MRSA, *A. faecalis*, *Provindecia. vermicola,* antimicrobial.

Activités antimicrobiennes des bactéries lactiques contre *Pseudomonas aeruginosa*, *Providencia vermicola*, *Alcaligenes faecalis* et *S. aureus* résistant à la méthicilline.

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RESUME

Contexte: Il existe une émergence rapide de pseudo-monades et de souches entero-bactériennes de pharmaco-résistants *S. aureus* (staphylococcus doré) résistant à la méthicilline (SARM), mais peu d'informations sont disponibles sur les effets des bactéries lactiques sur certaines souches d'enterobacteriaceae.

Objectif: Cette étude a consisté à évaluer l'effet antimicrobien de certaines souches de lactobacilles et de *Weisella* contre certains agents pathogènes potentiels sélectionnés (*Pseudomonas aeruginosa*, SARM, *Providencia vermicola* et *Alcaligenes faecalis*) en co-culture.

Méthodes: La susceptibilité aux antibiotiques des agents pathogènes potentiels sélectionnés a été réalisée par méthode de diffusion du disque et le surnageant sans cellules de quatorze souches différentes de bactéries lactiques a d'abord été testé contre les pathogènes potentiels sélectionnés par méthode de diffusion d'agar ... Les effets antimicrobiens de *Lactobacillus plantarum* 9, *Lactobacillus buchneri* SM04, *Lactobacillus fermentum* 008, *Lactobacillus brevis* 21 et *Weissella paramesenteroides* BS03 ont été testés contre les quatre pathogènes potentiels sélectionnés par deux méthodes de co-culture différentes à différents temps de contact.

Résultats: Les agents pathogènes potentiels testés étaient généralement résistants aux antibiotiques testés avec *Providencia. vermicola* présentant une résistance à 100%. *Lactobacillus brevis* 21, *Lactobacillus buchneri* SM04, *Lactobacillus plantarum* 9, *Lactobacillus fermentum* 008 et *Weissella paramesenteroides* BS03 ont des activités antimicrobiennes contre les agents pathogènes potentiels testés dans une expérience surnageant sans cellule. Les cinq bactéries lactiques testées ont inhibé les agents pathogènes potentiels en co-culture mais à un temps de contact différent. *Lactobacillus fermentum* 008 a totalement inhibé la croissance de *Providencia vermicola* après 18 heures de co-culture alors que tous les agents pathogènes potentiels ont été inhibés dans la culture de bactéries lactiques faite pendant la nuit.

Conclusion: Les bactéries lactiques ont de grandes activités antagonistes contre les agents pathogènes potentiels testés utilisés dans cette étude. Ainsi, elles ont de grands potentiels en tant que thérapie alternative dans les cas où la résistance aux antibiotiques a été établie.

Mots-clés: Lactobacillus, résistance, MRSA, *A. faecalis*, *Provindecia. vermicola*, antimicrobien.

INTRODUCTION

Infectious diseases are caused majorly by pathogens. The Enterobacteriaceae family include pathogens such as *Providencia vermicola, Pseudomonas aeruginosa, Alcaligenes faecalis, E. coli, Salmonella, Yersinia enterocolitica, Shigella spp, and Cronobacter spp.* Enterobacteriaceae have emerged to become pathogenic as a result of the acquisition of virulence associated genes which is known to be carried on mobile genetic materials such as plasmids.[1] Members of the family are responsible for causing foodborne disease and some also cause food spoilage and therefore contribute to substantial economic losses and food wastage. *Alcaligenes faecalis* has been reported to cause sporadic cases of endocarditis, meningitis, chronic otitis, pyelonephritis, bacteremia, endophthalmitis, and abscesses.[2,3] Treatment of infections caused by *A. faecalis* is often difficult because of high level of antibiotic resistance and trimethoprim/sulphamethoxazole are considered the agents of choice for treatment.[2] Daniel *et al.,*[4] reported *Acaligenes faecalis* as the unusual cause of skin and soft tissue infections. Infections due to *A. faecalis* are opportunistic and they are acquired from moist items such as nebulizers, respirators, and lavage fluids.

Bacteria belonging to the genus *Providencia* are responsible for human infections such as urinary tract infections, endocarditis, ocular infections, traveler's diarrhea, and gastroenteritis.[5] *Providencia vermicola* has been described as the possible cause of diarrhea in travelers and in children in developing countries.[6] Myonsun *et a*l.,[7] confirmed the importance of *Providencia* species as a cause of travelers'diarrhoea. *Providencia vermicola* is known as fish pathogen.[8]

Pseudomonas aeruginosa has described as an emerging pathogen in infective endocarditis.[9] *Pseudomonas aeruginosa* is associated with acute infections when normal host defenses are impaired or when extensive tissue damage has occurred.[10.] *Pseudomonas aeruginosa* has been known for years to be a cause of serious surgical infections and wounds; it is also regarded as a secondary or opportunistic pathogen rather than a cause of primary infection in healthy hosts, occurring in wide range of infections and observed antibiotic resistance.[11]

Staphylococcus aureus infections were sensitive to ß-lactam antibiotics but the emergence of Methicillin-resistant *S. aureus* (MRSA) has posed a serious therapeutic challenge. MRSA are pathogenic in nature, been implicated in a number of staphylococcal infections and possess a great variety of virulence factors. MRSA is a serious threat to hospitalized patients globally as well as to the community at large because of its general resistance to many other chemotherapeutic agents.[12]

The rapid emergence of drug-resistant strains, antibiotic cost and chronic toxicity[13,14] following the widespread use of antibiotics encourages us to study alternative treatment for bacterial infections. Lactic acid bacteria (LAB) are renowned for the potential of producing antimicrobial compound and other value added products.[15] There are many strains of LAB that show antimicrobial properties against a wide range of pathogen. Over the years research work has been done on the antimicrobial properties of LAB.[16-18] Their antimicrobial properties are based majorly on the substances that are secreted by LAB (e.g. organic acids, bacteriocins, hydrogen peroxide) and the viable cells.[16-17]

The vast antimicrobial potentials of probiotic organisms informed the urge to study the antimicrobial effect of LAB against pathogenic enterobacteriaceae and MRSA. There is little information on activities of LAB against *Pseudomonas aeroginosa, Providencia vermicola* and *Alcaligenes faecalis.* The aim of this study is therefore to determine the antimicrobial activities of LAB against multidrug resistant strains of Pseudomonas *aeroginosa, Providencia vermicola, Alcaligenes faecalis* and MRSA in co-culture.

METHOD

Bacterial Culture

Fourteen LAB species and four potential pathogens from our laboratory culture collection were used in this study. The potential pathogens are *Pseudomonas aeroginosa,* Methicillin resistant *Staphylococcus aureus* FAA091*, Providencia vermicola* FAA009 and *Alcaligenes faecalis* FAA008. The test strains were previously isolated from human nasal cavity (MRSA) and the human urogenital region (*Pseudomonas aeroginosa, Providencia vermicola* and *Alcaligenes faecalis*). The 3 strains isolated from urogenital region were selected because of their multidrug resistance status while MRSA was used because of the global concern associated with it. The LAB are: *Lactobacillus fermentum* (n=4)*, Lactobacillus plantarum* (n=4)*, Lactobacillus brevis* (n=1)*,Weissella paramesenteroides* (n=2), *Lactobacillus parabuchneri* (n=2) and *Lactobacillus buchneri* (n=1) . The 5 selected LAB strains for coculture are *Lactobacillus plantarum* 9, *Lactobacillus fermentum* 008*, Weissella paramesenteroides* BS03*, Lactobacillus brevis* 21, and *Lactobacillus buchneri* SM04.

Lactobacillus buchneri SM04 and *W.*

paramesenteroides BS03 has been previously isolated from yoghurt while L. *plantarum* 9, *L. fermentum* 008, and *L. brevis* 21 were isolated from human vagina.

Antibiotic Susceptibility Test

The antibiotic resistance pattern of the four potential pathogens used in this study was confirmed by disc diffusion method. The following antibiotic discs (Oxoid, UK**)** were used: Gentamicin (30µg), Kanamycin (30µg), Erythromycin (15µg), Penicillin (10µg), Vancomycin (30µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Neomycin (30µg), Sulphamethazole/Trimethoprim (23.73µg/1.25µg), Amoxicillin (10µg), Ampicillin (10µg), and Tetracycline (30µg). Liquid suspension of bacteria equivalent to 0.5 MacFarland standard were streaked on Nutrient agar plates according to standard techniques. The antibiotics discs were aseptically applied on surface of the medium. The plates were incubated at 37°C for 24 hours; after which they were examined for zones of inhibition. The breakpoint was interpreted according to Clinical and Laboratory Standards Institute breakpoint.[19]

Determination of Antimicrobial Activities of LAB Supernatant

Antimicrobial activities of Cell Free Culture Supernatant (CFCS) of 14 different LAB were initially screened to select five different LAB strains that would show good inhibitory effect on the four tested pathogens.

The LAB were individually grown in 10 mL MRS broth (Oxoid, UK) for 24 hours under microaerophilic condition. The CFCS were obtained through centrifugation for each LAB (12,000 g for 10 mins). The antimicrobial activities of the CFCS were determined by an agar well diffusion assay. Aliquots (30 µl) of the CFCS were placed in wells (7-mm diameter), bored in cooled Nutrient agar plates initially seeded with each potential pathogens. The plates were incubated for 24 hours at a 37°C and the zones of inhibition were measured in millimeter.[16,20]

Co-culture of LAB and the Pathogens

Two separate methods were employed in the determination of the effect of each LAB strain against each of the tested pathogens. The interference of each selected viable LAB with the growth of the potential pathogens was evaluated by co-incubating *Pseudomonas aeruginosa,* MRSA*, Providencia vermicola, and Alcaligenes faecalis* individually with each selected viable LAB strains (*Lactobacillus plantarum* 9, *Lactobacillus buchneri* SM04, *Lactobacillus fermentum* 008, *Lactobacillus brevis* 21, and *Weissella paramesenteroides* BS03).

The method of Drago *et al*.,[21] was used for the two co-culture experiments. In the first experiment, tubes containing 5 mL of MRS broth and 5 mL of Nutrient broth were inoculated with 0.1ml of both the selected viable LAB strain and the pathogen respectively. The Nutrient and MRS broths were prepared as double strength. The inoculated Nutrient and MRS broths were mixed thoroughly and plated out at appropriate dilutions to determine initial cfu/ml of the potential pathogens. There were controls (i.e. monocultures) for each of the selected viable LAB and the potential pathogens. The co cultures were plated out for potential pathogens and LAB separately on Nutrient agar, Mannitol salt agar and MRS agar respectively at o h, 6 h, 12h, 18 h, 24 h, 48 h, 72h hours. The controls were plated out initially (o h) and 6 h, 12h, 18 h, 24 h, 48 h, 72h in 15ml of nutrient agar except *Staphylococcus aureus* which was plated out on Mannitol Salt agar. The LAB controls were incubated in microaerophilic conditions while the potential pathogen controls were incubated in aerobic conditions. The surviving cells were counted to determine the cfu/ml of the potential pathogens.

The second experiment is similar to the first series of experiments except that the potential pathogens were inoculated in overnight cultures of the selected viable LAB. 0.1ml of the selected viable LAB was inoculated in MRS broth and was incubated at 37°C for 24 hours. After which 0.1ml of the potential pathogens were added into overnight 24 hours cultures of LAB. The mixture was plated out at time 0 hr then 24 hr at appropriate dilution. The surviving cells were counted to determine the cfu/ml of the potential pathogens.

RESULTS

Providencia vermicola exhibited 100% resistance to all the antibiotics tested while *Alcaligenes faecalis* showed 58.3% resistance to all tested antibiotics. *Pseudomonas aeruginosa* showed 50% resistance to all tested antibiotics. The tested MRSA was generally susceptible to tested antibiotics (75%) but resistant to penicillin and amoxillicin. (Table 1).

The CFCS of the LAB were generally active against all tested potential pathogens. *Providencia vermicola* has the largest zone of inhibition (17mm) with *Lactobacillus buchneri* SM04. For MRSA*,* the largest zone of inhibition was 13mm with *Lactobacillus plantarum* 9 *Pseudomonas aeruginosa* had the highest zone of inhibition of 15mm with *Weissella paramesenteroides* BS03. *Alcaligenes faecalis,* had the largest zone of inhibition (21mm) with *Lactobacillus plantarum* 9 (Table 2).

Co-culture experiments were performed on the potential pathogens and the selected viable LAB. *Weissella paramesenteroides* BS03 and *Lactobacillus plantarum* 9 totally inhibited the growth of *Providencia vermicola* after 24 hours of co-culture while *Lactobacillus fermentum* 008 totally inhibited the growth of *Providencia vermicola* after 18 hours of co-culture. *Lactobacillus fermentum* 008 and *Lactobacillus plantarum* 9 totally inhibited the growth of *A. faecalis* after 24 hours while *Weissella paramesenteroides* BS03 and *Lactobacillus brevis* 21 totally inhibited the growth of *A. faecalis* after 48 hours of co-culture. *Lactobacillus fermentum* 008 and *Lactobacillus buchneri* SM04 totally inhibited the growth of *Pseudomonas aeruginosa* after 24 hours of co-culture. *Lactobacillus fermentum* 008 and *Lactobacillus plantarum* 9 totally inhibited the growth of MRSA after 72 hours of co-culture) Figs I-IV).
In the second experiment that involves growing pathogens in overnight culture of LAB, all the pathogens were totally inhibited after 24 h (Fig V).

Table 1:Antibiotics Resistance of Potential Pathogens

Pathogens	GEN (mm	KAN (mm	ERY (mm	PEN (mm	VAN (mm	CHL (mm)	CPR (mm	SEP (mm)	AMO (mm)	AMP (mm)	TET (mm	NEO (mm)	%R
P. aeroginosa	S	R	R	R	R	S	S	S	S	R	S	R	50
MRSA	S	S	S	R	S	S	S	I	S	R	S	S	25
A. faecalis	R	R	R	R	R	R	S	R	S	S	S	S	58
Pro.vermicola	R	R	R	R	R	R	R	R	R	R	R	R	100

Key: (-) = S = Sensitive, R = Resistant, I = Intermediate
GEN=Gentamicin, KAN=Kanamycin, ERY=Erythromycin, PEN=Penicillin, VAN=Vancomycin, CHL=Chloramphenicol, CPR=Ciprofloxacin), NEO=Neomycin,, SEP=Sulphamethazole/Trimethoprim, AMO=Amoxicillin), AMP=Ampicillin, and TET=Tetracycline.

Table II. Antibacterial Activities of CFCS of Viable LAB against Tested Potential Pathogens.

			Zones of Inhibition (mm)	
Lactic acid bacteria	*Providencia vermicola* (mm)	MRSA (mm)	*Pseudomonas aeroginosa* (mm)	*Alcaligenes faecalis* (mm)
Lactobacillus fermentum 002	14	0	12	20
Lactobacillus fermentum 008	0	11	13	15
Lactobacillus fermentum 018	13	0	0	15
Lactobacillus fermentum 019	12	0	0	0
Lactobacillus plantarum 7	17	0	16	15
Lactobacillus plantarum 9	13	13	11	21
Lactobacillus plantarum 10	14	0	0	16
Lactobacillus plantarum 11	18	0	16	16
Lactobacillus brevis 21	0	0	0	19
Weissella paramesenteroides BS02	0	0	0	13
Weissella paramesenteroides BS03	15	0	0	0
Lactobacillus parabuchneri SM01	0	0	15	0
Lactobacillus parabuchneri SM03	0	0	0	0
Lactobacillus buchneri SM04	17	**0**	14	15

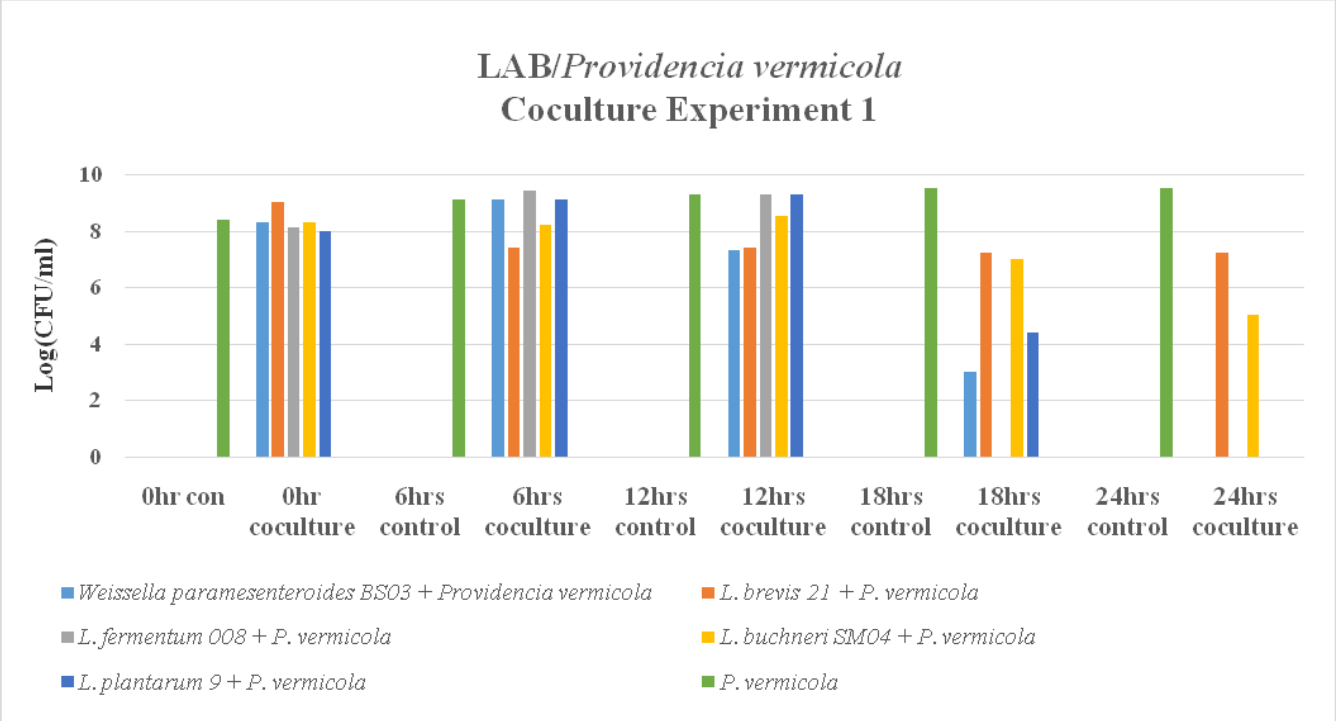

Figure I: Inhibition of *in vitro* growth of *Providencia vermicola* by co-incubating with LAB.

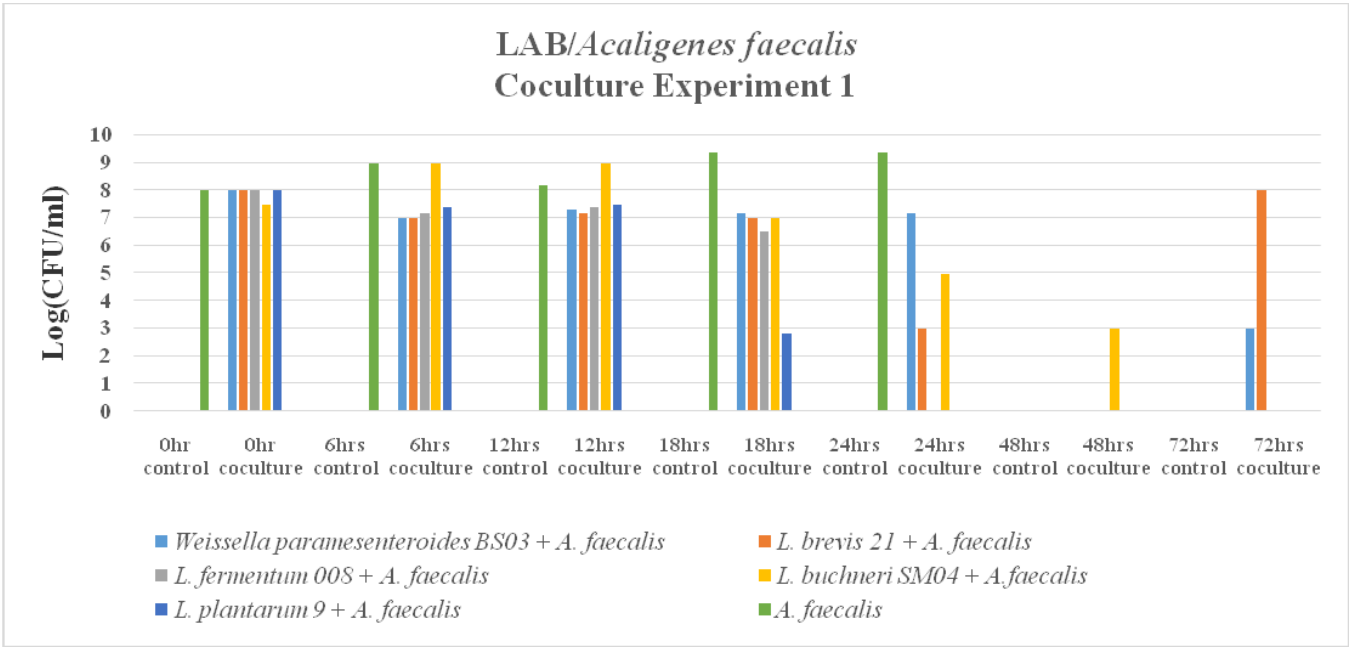

Figure II: Inhibition of *in vitro* growth of *A. faecalis* by co-incubating with LAB

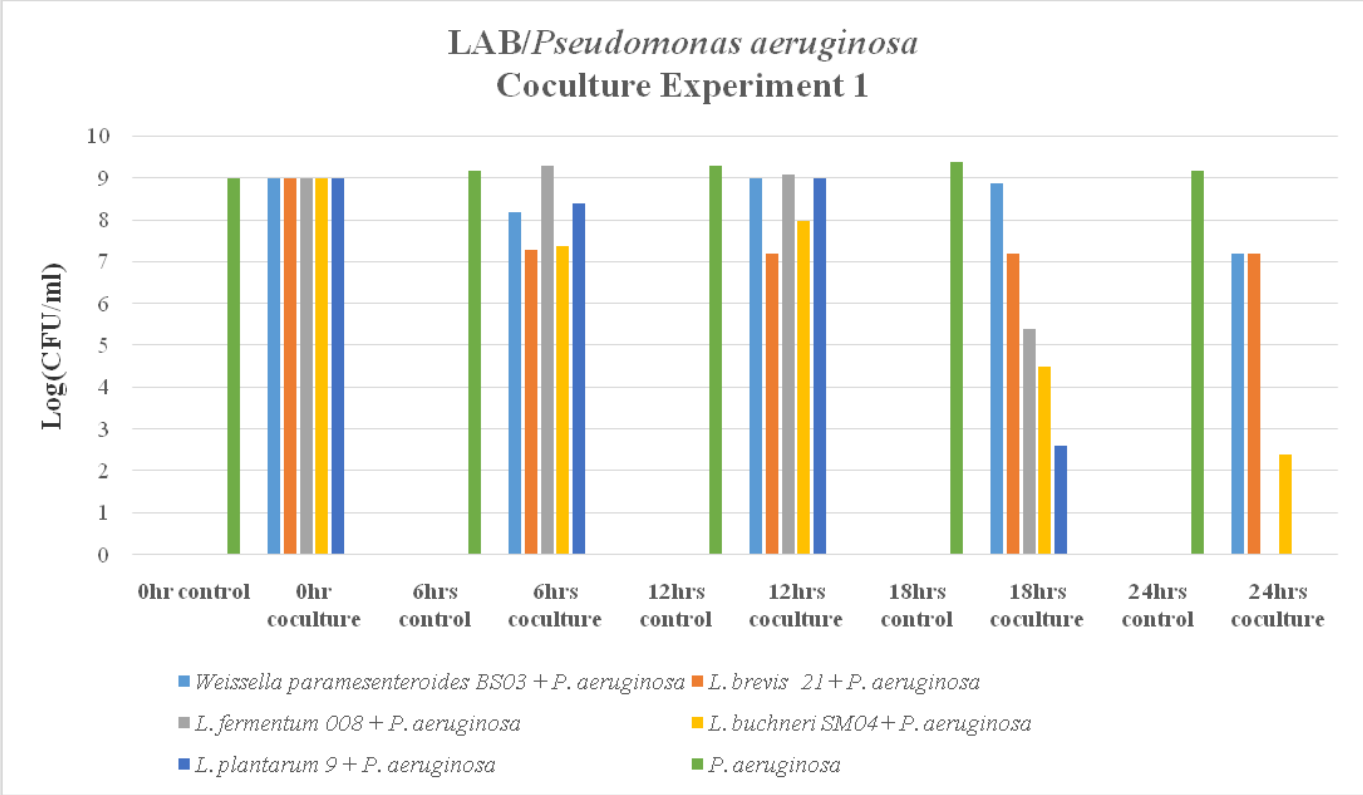

Figure III: Inhibition of *in vitro* growth of *Pseudomonas aeruginosa* by co-incubating with LAB.

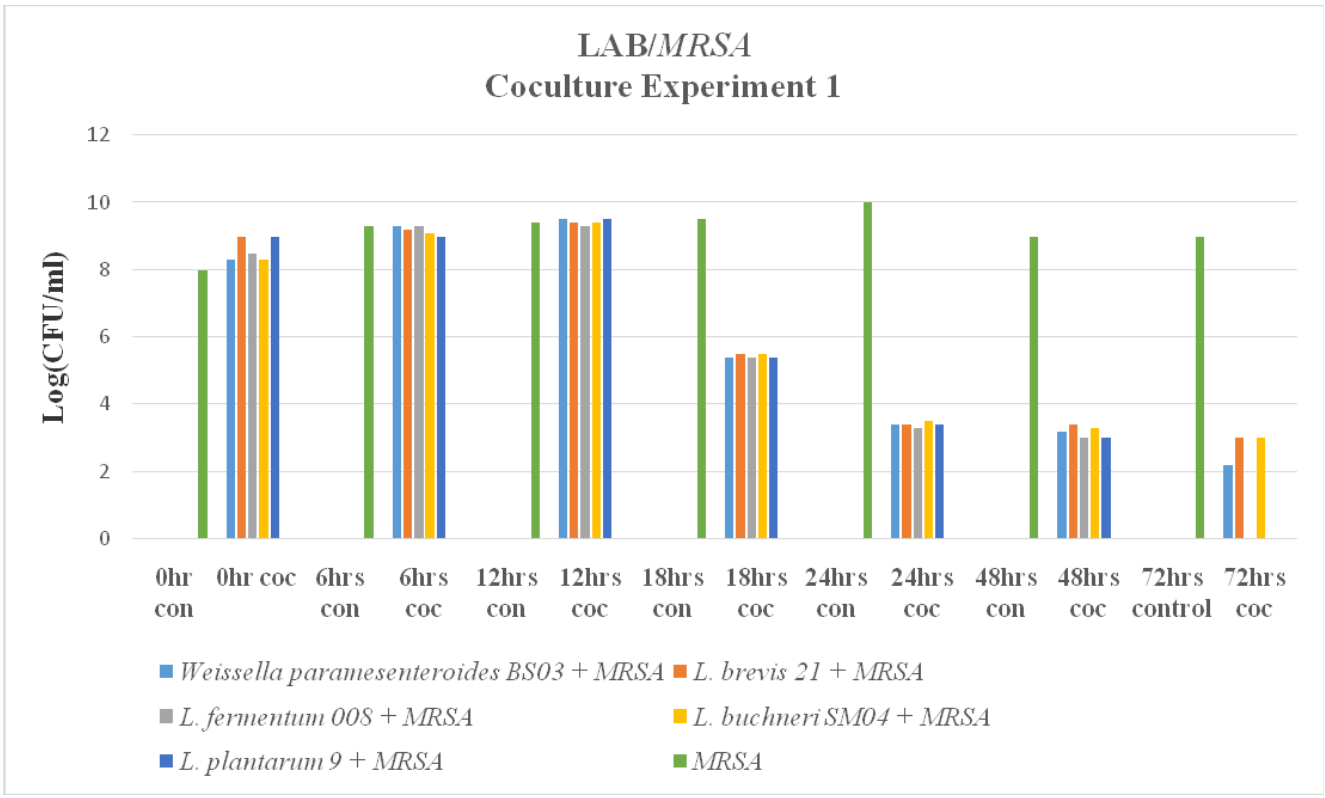

Figure IV: Inhibition of *in vitro* growth of MRSA by co-incubating with LAB.

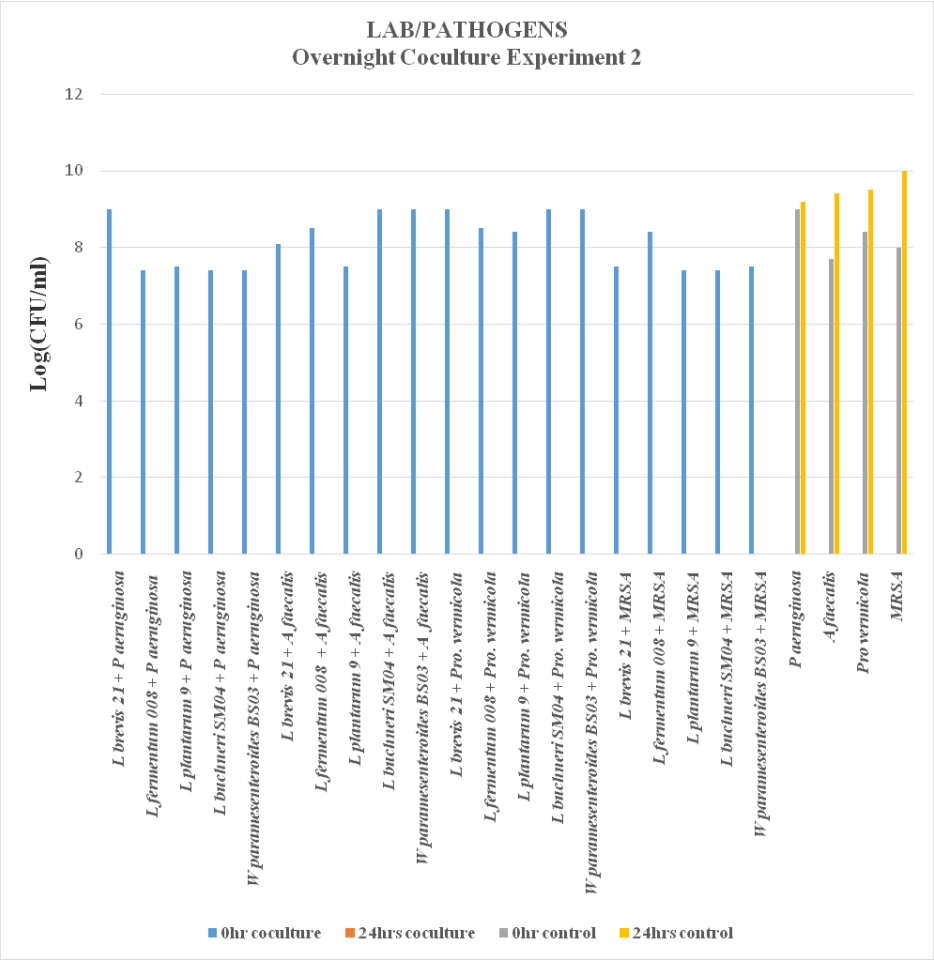

Figure V: Inhibition of *in vitro* growth of tested strains by growing potential pathogens in overnight culture of LAB.

DISCUSSION

Antibiotic resistance menace is a global problem that man battles daily. In this study, the tested *Alcaligenes faecalis* and *Pseudomonas aeruginosa* strains were resistant to at least 50% of tested antibiotics. *Pseudomonas aeruginosa* has a remarkable capacity to resist antibiotics, intrinsically or by acquisition of resistance genes.[22] The tested *Providencia vermicola* was not susceptible but resistant to all eleven antibiotics tested against it. This represents 100% resistance which would pose a big problem in the healthcare system. In the study conducted by Neha *et al*.,[23] where *Providencia vermicola* was investigated for genetic elements governing their drug resistance phenotypes, *Providencia vermicola* was found to be resistant to 14 antibiotics except polymyxin B. This corresponds to the results of our studies. Hleba *et al*.,[24] conducted a study on monitoring of antibiotic resistance of *Enterobacteriaceae* genera isolated from rectal swabs of ducks during seven weeks interval. The results obtained showed that the highest resistance of *Enterobacteriaceae* was to tetracycline (32.43%) while streptomycin and ampicillin were 8.10% and chloramphenicol was 5.40%. Antibiotics resistant bacteria are a biological danger in relation to animal and human health. Gram-negative enterobacteriaceae may cause infections that are severe and also species of this family are become progressively resistant to a wide range of antimicrobials used against them.[25] Azevedo *et al.,*[26] reported existence of antibiotic resistant enterobacteriaceae in the domestic food related environments in which 49.6% of the isolates were resistant to at least one antibiotics.

With the apparent positive success of probiotic products in the developed parts of the world, interests concerning the nature of probiotic bacteria, their potential health effects and benefits are generated.[27] Supayang *et al.,*[28] reported activities of culture supernatant of *Lactobacillus reuteri* against MRSA. The antibacterial ability of *Lactobacillus reuteri* was fundamentally attributed to their bacteriocin

production which can cause both cell inhibition and cell death.

In this study, most of the tested pathogens tested were also susceptible to the CFCS of the various LAB but with varying degree of susceptibility. *Alcaligenes faecalis* has the highest susceptibility while MRSA was the least susceptible. Mariam *et al*.,[29] evaluated the potential of CFCS from cultures of selected lactic acid bacteria and yeast obtained from local fermented foods as inhibitors of *Listeria monocytogenes, Salmonella spp,* and *Staphylococcus aureus*. The CFCS proportion-dependent progressively decrease the number of colonies and also both growth rates and number of generations were reduced. Ayeni *et al.,*[16] also reported bacteriocin like substance in CFCS of LAB isolated from Nigeria.

From the co-culture experiment, all the tested pathogens were completely eradicated after a specific period of time and there were log reductions in the viable count of the pathogen used at different time intervals between 2 -9 log reductions with all tested LAB. Cheng-Chih *et al*.,[30] reported inhibition of *E. coli* growth by *Lactobacillus* strains while Asha and Gayathri[31] also confirmed the effectiveness of the *Lactobacillus spp* against enteropathogenic bacteria and Drago *et al*., [21] reported a co-culture study in which *Lactobacillus* strains inhibited the *in vitro* growth of *E. coli, Salmonella enteriditis* and that a cumulative effect was observed when the *Lactobacilli* were co-cultured with any of the pathogens. Szala *et al.,*[32] co-cultured six *Lactobacillus* strains with *Salmonella senftenberg* and gave a report that all the tested trains inactivated the growth of the test pathogen during a 48 hours co-culture and we also previously reported reduction in *E. coli* counts in co-culture with lactobacilli and other antagonistic activities.[33,34] Interestingly, the present study also reports inability of tested potential pathogens to grow in overnight cultures of LAB. This may find application in prophylaxis cases where LAB is already established in human and being able to totally repel pathogens attack. The present study only analyzes antimicrobial activities of lactic acid bacteria in co-culture. Further studies are necessary to explore other mechanisms of actions.

CONCLUSION

Our tested LAB strains are effective in inhibiting the growth of *Pseudomonas aeruginosa, Providencia vermicola, Alcaligenes faecalis,* and MRSA *invitro* thus having great potentials of applications in infectious states. These results further affirm the fact that lactic acid bacteria can be an alternative treatment option in cases where conventional antibiotics are no longer effective due majorly to resistance of pathogenic organisms to these antibiotics. Further *invivo* studies are needed to explore these potentials.

ACKNOWLEDGEMENTS

We acknowledge all the technical staff at Department of Pharmaceutical Microbiology, University of Ibadan, Nigeria.

REFERENCES

1 Baylis C, Uyttendaele M, Joosten H, Davies A (2011). The Enterobacteriaceae and their significance to the food industry. International Life Science Institute. *Europe Emerging Microbial Issues Task Force* 1-50.

2 Kavuncuoglu F, Unal A, Oguzhan N, Tokgoz B, Oymak O, Utas C. (2010). First reported case of *Alcaligenes faecalis* peritonitis. *Peritoneal Dialysis International* 30:118-119.

3. Khokhar D.S, Sethi H.S, Kumar H, Sudan R, Sharma N, Nayak N (2002). Post-keratoplasty endophthalmitis by *Alcaligenes faecalis. Cornea* 21:232-3.

4 Daniel T, Cristina F, Maria RL (2015). *Alcaligenes faecalis*: an unusual cause of skin and soft tissue infection. *Japanese Journal of Infectious Disease* 68: 128-130.

5. Koreishi AF, Schechter BA, Karp CL (2006). Ocular infections caused by *Providencia rettgeri*. *Ophthalmology* 113: 1463–1466.

6. Albert MJ, Faruque ASG, Mahalanabis D (1998). Association of *Providencia alcalifaciens* with diarrhea in children. *Journal of Clinical Microbiology* 36: 1433–1435.

7. Myonsun Y, Junko M, Motoki O, Kazuhiro T, Hirozane M, Kazuhiro M, Kwon-Sam P, Takahiro O, Takeshi H (2005). Importance of *Providencia* species as a major cause of travellers' diarrhea. *Journal of Medical Microbiology* 54: 1077–1082

8. Balasubramani G, Deepak P, Sowmiya R, Ramkumar R, Perumal P (2015) Antigononleptopus: a potent biological source for extermination of fish bacterial pathogens *Providencia* and *Aeromonas*. *Natural product research* 29(10):958-960.

9 Gul S, Eraj A, Ashraf Z (2014). *Pseudomonas aeruginosa*: A common causative agent of ear infections in South Asian children. *International Journal of Current Microbiology and Applied Science* 3(6): 156-160.

10 Bodey GP, Jadeja L, Elting L. (1985). *Pseudomonas*

bacteremia. Retrospective analysis of 4 episodes. *Archives of Internal Medicine* 145:1621-1629.

11. Iregbu KC, Eze SO (2015). *Pseudomonas aeruginosa* Infections in a Tertiary Hospital in Nigeria. *Afrian Journal of Clinical and Experimental Microbiology*. 16 (1).
12 Ayeni FA, Olatunji DF, Ogunniran M (2014). Prevalence of methicillin resistant *Staphylococcus aureus* and resistance pattern of its clinical strains to beta-lactam antibiotics. *African Journal of Biomedical Research* 17: 129-133.
13 Cetinkaya Y, Falk P, Mayhall CG (2000). Vancomycin-resistant enterococci. *Clinical Microbiology Review* 13: 686-707.
14 Mody L, Kauffman CA, McNeil SA, Galecka AT, Bradley SF (2003). Mupirocin-based decolorization of Staphlococcus aureus carriers in residents of 2 long-term care facilities: a randomized, double-blind, placebo controlled trial. *Clinical Infectious Disease* 37: 1467-1474.
15. Khalisanni K. An overview of lactic acid bacteria (2001). *International Journal of Bioscience* 1(3): 1-13.
16. Ayeni FA, Adeniyi BA, Ogunbanwo ST, Tabasco R, Paarup T, Peláez C. Requena T (2009). Inhibition of uropathogens by lactic acid bacteria isolated from dairy foods and cow's intestine in western Nigeria. *Achieves of Microbiology* 191: 8: 639-648.
17. Ayeni FA, Sánchez B, Adeniyi BA, de los Reyes-Gavilán CG, Margolles A. Ruas-Madiedo P (2011). Evaluation of the functional potential of Weissella and Lactobacillus isolates obtained from Nigerian traditional fermented foods and cow´s intestine. *International Journal of Food Microbiology* 147. 2: 97-104.
18. Ayeni FA, Adeniyi BA (2013). Antimicrobial potentials of lactic acid bacteria isolated from a Nigerian menstruating woman. *TAF Preventive Medicine Bulletin.* 12.:3: 283-290.
19. Clinical and Laboratory Standards Institute (CLSI) (2014). Performance standards for antimicrobial susceptibility testing; 15th informational supplement. CLSI/NCCLS M100-S15. Clinical and Laboratory Standards Institute, Wayne, Pa.
20 Perez PF, De Antoni GL, Anon MC (1991). Formiate production by *Streptococcus thermophilus* cultures. *Journal of Dairy Science* 74: 2850-2854.
21 Drago L, Gismondo MR, Lombardi A, le Haen C. Gozzin L (1997). Inhibition of in vitro growth of enteropathogens by new *Lactobacillus* isolates of human intestinal origin. *FEMS Microbiology Letters* 153: 455-463.
22 Mesaros N, Nordmann Plesiat P, Roussel-Delvallez., Van Eldere J., Glupczynski Y., Van Laethem Y, Jacobs F, Lebecque P, Malfroot A, Tulkens PM, Van Bambeke F (2007). *Pseudomonas aeruginosa*: resistance and therapeutic options at the turn of the new millennium. *European Society of Clinical Microbiology* and *Infections* 13:560-578.
23 Neha R, Braj MR. Kutar NS, Sinha R, Nag D, Koley H, Ramamurthy T, Bhardwaj AK (2015). Role of integrons, plasmids and SXT elements in multidrug resistance of *Vibrio csholerae* and *Providencia vermicola* obtained from a clinical isolate of diarrhea. *Frontiers in Microbiology* 6: 57.
24 Hleba L, Kačániová M, Pochop J, Lejková J, Čuboň J, Kunová S (2011). Antibiotic resistance of Enterobacteriaceae genera and *Salmonella* spp., *Salmonella entericaser*. Typhimurium and Enteriditis isolated from milk, cheese and other dairy products from conventional farm in Slovakia. *Journal of Microbiology, Biotechnology and Food Sciences* 1: 1-20.
25 Denton M. Enterobacteriaceae (2007). *International Journal of Antimicrobial Agents* 29: S9-S22.
26. Azevedo I, Albano H, Silva J, Teixeira P (2015). Antibiotic resistance of Enterobacteriaceae isolated from the domestic food related environments. *Journal of Food quality and hazards control* 2: 51-55.
27. Salminen S, Isolauri E, Salminen E (1996). Clinical uses of probiotics for stabilizing the gut mucosal barrier: successful strains and future challenges. *Antonie Van Leeuwenhoek* 70:347-58.
28 Supayang PV, Sopa B, Orawan S (2006). Antagonistic activity against pathogenic bacteria by human vaginal lactobacilli. *Anaerobe* 12(5-6):221-226.
29. Mariam SH, Zegeye N, Tariku T, Andargie E, Endalafer N, Aseffa A (2014). Potential of cell-free supernatants from cultures of selected lactic acid bacteria and yeast obtained from local fermented foods as inhibitors of *Listeria monocytogenes, Salmonella* spp and *Staphylococcus aureus. BMC Research Notes* 7:606.
30. Cheng-Chih T, Pei-Pei L, You-Miin H (2007). Three *Lactobacillus* strains from healthy infant stool inhibit enterotoxigenic *Escherichia coli* grown in vitro. *Anaerobe* 14: 61-67.
31. Asha CS, Gayathri D (2012). Antagonistic Potential of Lactobacillus spp against Enteropathogenic Bacteria; Purification and Characterization of their

Bacteriocins. *Advance Journal of Food Science and Technology* 4(5): 265-269.

32. Szala B, Paluszak Z, Motyl I (2012). Antagonistic Effect of LAB on Salmonella seftenberg in Mixed Cultures. Polish Journal of Environmental Studies 21(5): 1399-1403.
33. Afolayan AO, Ayeni FA (2017). Antagonistic effects of three lactic acid bacterial strains isolated from Nigerian indigenous fermented *Ogi* on *E. coli* EKT004 in co-culture. *Acta Alimentaria, An International Journal of Food Science* 46(1) 1–8.
34. Afolayan AO, Ayeni FA, Ruppitsch W (2017). Antagonistic and quantitative assessment of indigenous lactic acid bacteria in different varieties of ogi against gastrointestinal pathogens. *Pan African Medical Journal* 27:22.