

ACTIVITY ASSAY OF SOME PROBIOTICS EXTRACT AS ANTI-*Pseudomonas aeruginosa* THAT ISOLATED FROM POSTOPERATIVE WOUND INFECTION IN ALNAJAF GOVERNORATE -IRAQSuhad Mohammed^{*1}, Ruaa SH¹, Atheer Basil²

Address (es):

¹ University of kufa, Faculty, Laboratory of investigation department, Najaf, , +9647903762689² University of kufa, Faculty, Biology department, Najaf.*Corresponding author: suhadm.shiltagh@uokufa.edu.iq<https://doi.org/10.36547/be.2021.4.1.16-19>

ABSTRACT

Background and objectives: Probiotics was redefined as “live microorganisms administered in inadequate amount that confer a health profit on the human for that the aim of this study were possibility of using probiotics extract with some supporting substances in antibacterial that resistant antibiotics.

Materials and methods: The methods of this study included isolated and identification of bacteria from postoperative wound infection of 50 women subjected to surgical birth process then selected the *P.aeruginosa* isolates and scanning the sensitivity test of some cephalosporin type of antibiotics and determine the presence of blaOXA-1 gene, also the study had tested activity of two types of probiotics (*Bacillus clausii* and *Saccharomyces boulardii*) extract with some supporting substances (acetic acid and citric acid) as antibacterial.

Results: The results of identification of bacterial isolates showed that 22(44%) isolates that related to *Pseudomonas aeruginosa*, 5(10%) isolates related to *E.coli*, 10(20%) isolates related to *Klitsella spp.*, 10(20%) isolates related to *Staphylococcus aureus* and 3(6%) isolated related to *Protus mirabils*, and Overall, 22 isolates of *P.aeruginosa* were analyzed by Kirby Bauer disk diffusion method and notice the resistance or sensitive to cephalaxin, ceftriaxon and cefpime where the results confirmed 14 cephalosporin resistant isolates of *Pseudomonas aeruginosa*. Also detect blaOXA-1 gene by genotypic test. Amplification of b-lactamase genes shows the presence of blaOXA-1 (at 427 bp line) on among (10/22) study isolates. The results of antibacterial activity showed that inhibition zone of probiotics extract only (14 and 16)mm to *S.bouldarii* and *B.clausii* respectively but it showed synergism effect when supporting the *S.bouldarii* and *B.clausii* extract with acetic acid where (18 and 20.3)mm respectively while (19 and 23.5)mm with citric acid respectively with significantly increasing differences when subjecting the bacteria to cefime ,ceftriaxone and cephalaxin antibiotics so, the inhibition zones were (1.3, 10.3 and 0)mm respectively.

Conclusion : In light of the foregoing, we concluded the possibility of using probiotic extract with some supported substances as an anti-bacterial *Pseudomonas aeruginosa* isolated from surgical wound infection.

Keywords: Probiotics, postoperative infection, blaOXA-1, *Bacillus clausii* and *Saccharomyces boulardii*

INTRODUCTION

Postoperative injury disease or infection of surgical site is a significant reason for medical services related to infection in patients of surgical. Longer clinic stays of patients that create wound infection, extra costly hospitalizations, and expanded death (Kirkland *et al.*, 1999). The advancement of wound infection relies upon the respectability and defensive in skin functions (Calvin, 1998).

Pseudomonas aeruginosa may be a principal reason behind infection that health care relationship, classification second among negative gram microbes as informed by the United States national nosocomial infection surveillance system. *P. aeruginosa* donates well to morbidity and mortality by wound infection worldwide. Entering of microbes into blood stream, inflicting sepsis which may unfold to the skin and results in (ecthyma gangrenosum), a black lesion necrosis (Khan *et al.*, 2008). Current ways to forestall wound infections concentrate on decreasing exposure of tissues to bacterium, improving tissue integrity and defenses, and administering antibiotics. But the prevalence of antibiotics resistant among bacterium were spread wide particularly by *P.aeruginosa*. The resistance mechanism to β -lactam antibiotics of *P. aeruginosa* can be credited to the gaining of the β -lactamase gene such as the blaOXA-1 frequently has been found in plasmid and integron locations in an exceedingly many types of negative gram microbes. The blaOXA-1 sequence has often been found to be related to genes that encoded extended-spectrum β -lactamases (ESBLs). OXA-1 β -lactamase, similar most OXAs, can hydrolyze amino and ureidopenicillins (piperacillin) considerably and narrow-spectrum cephalosporins decrepit hydrolyzing. Additionally, the hydrolyzing of blaOXA-1 gene was broad-spectrum cephalosporins, reduced sensitivity conferring to both cefpirome and cefepime. Current studies have reportable terribly common relationship of blaOXA-1 with the worldwide-extent CTX-M-15 ESBL contributing factor establish among human *E.coli* isolates from various environmental origins. This relationship of blaOXA-1 with blaCTXM genes creates isolates resistant β -lactam- β -lactamase substance mixtures (Khan *et al.*, 2008). Studies showed by our cluster over the five years ago has revealed increasing ESBL proportion (Poirel *et al.*, 2011) with several co bearing of ESBL, Amp C and NDM enzymes. There aren't several Iraqi studies on blaOXA-1 gene, through there are some studies on blaOXA-1 gene at the side of association of ESBL (SHV, CTX-M and TEM) genes. For that reason, we have a tendency to tried to assess the occurrence of blaOXA-1 gene in our sets and detect an insilico approach that discovers the resistance mechanism by OXA1 β -lactamases.

In recent decades, researchers have turned to the utilization of alternatives to combat the development of microorganism resistance to antibiotics, an example of that is that the use of probiotics. The probiotics using to boost health of human has been planned for several years (Sanders, 2000). Lately, the word probiotics was redefined as “live microorganisms administered in inadequate amount that confer a health profit on the human (FAO/WHO, 2001). Probiotics will minimize the occurrence of sure infections and weaken symptoms of such infections. By exploitation probiotics the utilization of antibiotics is also reduced and therefore give to reduce or a postponement of the increase bacteria that multi antibiotics resistant. This increasing problem worldwide has the potential to bring on a harmful like scenario in newly healthcare. Furthermore, numerous researchers have earlier stated that antimicrobial activity of probiotic in vitro and in vivo is mostly credited to their capability to provide antimicrobial constituents. Current reports recommend that the antimicrobial activity of probiotics lactobacillus includes the assembly of compounds that secreting, corresponding to organic acid, hydrogen peroxide, moreover as varied antibiotics and/or bacteriocins (Vescovo *et al.*, 1993).

The objective of our study was possibility of using probiotics extract with some supporting substances in antibacterial that resistant antibiotics.

MATERIAL AND METHODS

Bacterial Specimens Collection

Fifty specimens of surgical wounds infection were collected from women patients who visit the gynecology clinic after a surgical birth process and had wound inflammation despite taking cephalosporin antibiotics, in Al- Najaf province.

Preparing bacterial media

All media preparing according to precautions of companies.

Cultivation of Clinical Specimens

The samples were cultured on MacConkey and blood agar, incubated at 37 °C for 24hrs.

Identification the isolates by Vitek-2 Compact (Bio Mérieux,France,) technique

Along with the manufacturer's recommendation suspension of bacteria was prepared. Collecting enough number of colonies after overnight growth pure culture transported and suspending in 3.0 ml of sterile saline in a 12 x 75 mm clear (polystyrene) test tube. Adjusting the turbidity with 0.5 McFarland. Turbidity meter called Densi-Chek.was used for that. Also in GN-ID with VITEK-2 compact system was applied same suspension. Lastly GN-ID cassette was overloaded to the vitek-2 chamber together with suspension tubes of specimen (Karagz et al.,2015).

Prevalence of blaOXA-1 gene in Pseudomonas aeruginosa isolates

One colony of bacteria isolate from a blood agar petridish was vaccinated into 5 ml of Nutrient broth media (BD, MD, USA) and incubated for 16–18 h at 37°C⁰. 1.5 ml of the 24 hours cell culture were harvested by centrifugation at 8000 rpm for 5 min. After removing the supernatant, the pellet re suspended in distilled water (500 ml). Then cells was destroyed by heating at 95°C⁰ (10 min), and debris of cells was withdrawn by centrifugation at 8000 rpm for 5 min. (2 ml) of supernatant was used as the source of DNA template for amplification. PCR was achieved with an ending volume of 25 ml in 0.2-ml thin-walled tubes. The sequencing primer were as follows: blaOXA-1 forward, 59 TTTTCTGTTGTTGGGTTT 39; blaOXA1 reverse, 59 TTCTTGGCTTTTATGCTTG 39 (Bert et al.,2002). Individually reaction enclosed 20 mM Tris-HCl (pH 8.4); 50 mM KCl; 0.2 mM each deoxynucleotide triphosphate; 1.5 mM MgCl₂; primers OXA-1 F, OXA-1 R, and 1.25 U of Taq DNA polymerase (Promega,USA). The PCR program comprised an initial denaturation step at 95°C for 2 min, followed by 30 cycles of DNA denaturation at 94°C for 45 s, primer annealing at 55°C for 30 s and extension 72°C⁰, 1 min (Bert et al.,2002). After the last cycle, a final extension step at 72°C for 5 min was added. Fifteen-microliter aliquots of PCR product were investigated by gel electrophoresis with 2% agarose (USB Corporation, Cleveland, USA). Gel were stained with ethidium bromide at 1.5 mg/ml and imagined by UV transillumination. A 100bp- 4kbp DNA ladder (Promega,USA) was used.

Probiotics sample

Bacillus clausii and *Saccharomyces boulardii* probiotics was gained from local pharmacy in Al-Najaf province. Both probiotics were cultivated brain hart infusion broth (DifcoLaboratory,Detroit,MI) or on MH agar for 24 to 72 hours at 30°C .

Extraction of probiotics extracellular substances

Both probiotics were cultivated in 400 ml of brain hart infusion broth for 48 h at 37c , growth media was filtered by 0.2 mm naglin filter membrane then concentrated at 40c ,then using the 200 µg/ml concentration at antagonistic study.

Preparation some supporting substances

Acetic acid and citric acid (GCC,England) (1%, 2%) respectively were prepared for adding to final concentration of each probiotics extracts.

Determination of Antimicrobial Activity

To detection the probiotic antimicrobial activity a colony overlay assay was used. The presence of antagonistic effect of the probiotics strains was firm as an inhibition the growth of bacteria around the tested substances spots. Amethod of agar well diffusion was used for antimicrobial activity detection (Holder& Boyce,1994). Susceptibility test was carried by prepared the Muller hinton agar media (Himedia, India) and poured it in plates and lefts to solidify then culturing the bacteria (the turbidity of bacteria must equaled with 0.5Mcferland tube) entire the petridish with swab spreading culture method after 15 minutes by using crock pours (must be sterilized) four wells were made in agar then filled with one substances as below:

Well diffusion method were applied on:

1-100 µl of *Saccharomyces boulardii* extract 200 µg/ml

2-100 µl of *Bacillus clausii* extract 200 µg/ml

3-(v/v) 100 µl of *Saccharomyces boulardii* extract 200 µg/ml and acetic acid (A.A) 1% or citric acid (C.A)2%

4-(v/v) 100 µl of *Bacillus clausii* extract 200 µg/ml and acetic acid (A.A) 1% or citric acid (C.A)2%

Disc diffusion method were applied on cephalixin, cefotriaxon and cepime antibiotics with (20 µg.disk-1) for each one.

We must not forget the labeling each well, all plates was put in incubator for 24 hrs.

Statistics analysis

All data were collected and analyzed by soft were SPSS version 24.

RESULTS AND DISCUSSION

Identification of Bacterial Isolates

Identification of bacterial isolates based on, cultured, morphological and vitek. The results showed that 22(44%) isolates that related to *Pseudomonas aeruginosa*, 5(10%) isolates related to *E.coli*, 10(20%) isolates related to *Klibella spp.* , 10(20%) isolates related to *Staphylococcus aureus* and 3(6%) isolated related to *Protus mirabils*.(Table 1) Many studies referred to many types of bacteria can be isolated from wound skin infection as (12) who showed that The most public bacterial species noticed was *Staphylococcus aureus* (37%), followed by *Pseudomonas aeruginosa* (17%), *Proteus mirabilis* (10%), *Escherichia coli* (6%) and *Corynebacterium spp.* (5%). The most one communal infections are Skin and soft tissue infections (SSTIs) of all age collections patients. Infections frequently are self-healing or antibiotics treating. Though, reasonable or severe cases may need hospitalization and parenteral treatment (Petkovšek et al.,2009). Surgical infections are generally belong to endogenous flora and some other ecological sources in the operating theater. The deep-seated sepsis emerging within 30 days after a surgery and before the wound has been dressed redirect a theater infection. Approximately of the studies support the concept that a decrease in postoperative wound infection is directly related to better education and awareness of its causes, and its prevention is greatly assisted by critically assessed infection control practice (Leigh et al.,1990).

Table 1 Distribution of pathogenic bacteria in postoperative wound infection

	<i>Pseudomonas aeruginosa</i>	<i>E. coli</i>	<i>Klebsiella spp</i>	<i>Staphylococcus aureus</i>	<i>Proteus mirabils</i>
50	22(44%)	5(10%)	10(20%)	10(20%)	3(6%)

Antibiotics susceptibility

Overall, 22 isolates of *P. aeruginosa* were analysed by Kirby Bauer disk diffusion method and notice the resistance or sensitive to cephalixin, cefotriaxon and cepime, noted (Table 2).

The results of Antibiotics susceptibility lined with (Bonfiglio et al.,1998) who showed that multidrug resistance for about 8 to 11 antibiotics was detected among the 55.5% of the bacterial isolates and the results of disk diffusion showed 100% resistance to ampicillin, cefoperazone, erythromycin, norfloxacin, and only

cefotaxime, ciprofloxacin presented better action against *Pseudomonas aeruginosa*.

The causes of these results may be related to the fact that the bacterial isolated from clinical specimens have been exposed to the selective activities of both disinfectants and antibiotics (Marseglia et al.,2007)

The inherent resistance mechanism of *P. aeruginosa* is one of the important challenges in management infections of this bacteria, that referred to as intrinsic resistance. Its multiplicity of resistance mechanisms may decrease this bacteria fewer amenable to regulator by antibiotic cycling. *P. aeruginosa* is distinguished for its metabolic flexibility and its exceptional capacity to colonize a wide

variation of environments and also for its intrinsic resistance to a widespread variety of antimicrobial agents. (Bonfiglio *et al.*,1998)

Table 2 Sensitivity test of 22 *P.aeruginosa* isolates with some types of cephalosporin antibiotics

Antibiotic	Isolate number																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
cephalexin	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	S	S	S
cefotriaxon	R	R	S	S	R	R	R	R	R	R	R	R	S	S	R	R	S	R	R	S	S	S
cefpime	R	R	S	R	R	R	R	R	R	R	R	S	R	S	R	R	S	R	R	S	S	R
R-resistant																						
S-sensitive																						

Molecular investigation of the presence of a beta-lactam resistance gene:

Phenotypically confirmed 14 cephalosporin resistant isolates of *Pseudomonas aeruginosa* are used to detect blaOXA-1 gene by genotypic test. Amplification of b-lactamase genes shows the presence of blaOXA-1 (at 427 bp line) on among (10/22) study isolates (Figure 1).

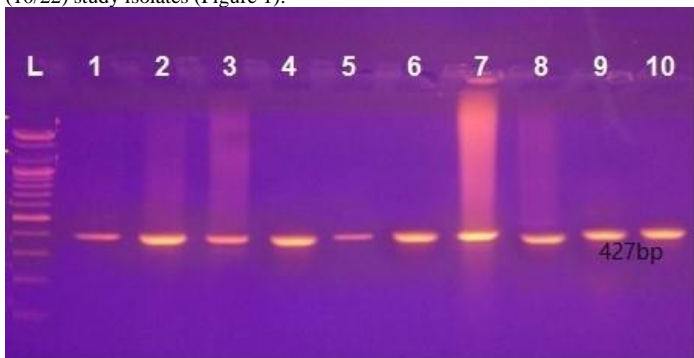


Figure 1 Agarose gel analysis of PCR profile obtained on Amplification with primers blaOXA-1 forward and blaOXA-1 reverse, specific for *P. aeruginosa* .lane(1 and 10): *P. aeruginosa*;(1,10) lane amplification of blaOXA-1 gene, lane(L) DNA Ladder 100-4kbp. (2% Agarose gel, 80 volts for 1 hour).

Antimicrobial activity assay

This assay included tested *S.bouldarii* and *B.clausii* extracts and supporting them with acetic and citric acid on *Pseudomonas aeruginosa* isolates then comparing it with three types of cephalosporin antibiotics. The results showed that inhibition zone of probiotics extract only (14 and 16mm to *S.bouldarii* and *B.clausii* respectively but it showed synergism effect when supporting the *S.bouldarii* and *B.clausii* extract with acetic acid where (18 and 20.3mm respectively while (19 and 23.5mm with citric acid respectively with significantly increasing differences when subjecting the bacteria to cefime ,ceftriaxone and cephalaxin antibiotics the inhibition zones were(1.3,10.3 and 0)mm respectively (Table3).

Antimicrobial activity assay of interpreted by the probiotics may interfere with microbe attack by decreasing or repressing microorganism adherence, carrying antimicrobials, or toxin interfering (Marseglia *et al.*,2007). For sure, a few microbes, for example, *C. difficile*, *B.cereus*, *Vibrio cholerae*, and *Escherichia coli*, may produce toxins that are involved in virulence of bacteria. Proteins secreted and delivered within environment may mediate some of the positive effects observed in probiotics (Imperial & Ibana ,2016).

Numerous investigations mentioned that Cell lysate of yeast demonstrated preferred antimicrobial action over entire cell and supernatant of culture. Once more, the isolate demonstrated better antibacterial action against gram negative than gram positive bacteria. Culture supernatant demonstrated minimum antibacterial action showing that the antibacterial compounds are cell bound but not extracellular secretions. (Rajkowska& Kunicka ,2012). Probiotic reports yeast strains (belonging to *S. cerevisiae* and *S. bouldarii*) which demonstrated inhibition effect against pathogen that infected human, for example, *Listeria monocytogenes*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis* (Roostita *et al.*,2011)

B.clausii probiotic strains which have Functional were exhibited that *B. clausii* discharges an antimicrobial substances active against *C. difficile*. Afterward, they purified and described, from culture supernatant, abacteriocin , the clausin

(Marseglia *et al.*,2007), that represses *C.difficile* resist conceivably valuable MICs (0.5 to 1 g/ml).

The resistant of spores to diverse types of antibiotics and antibiotics production or enzymes were secreted inside the canal intestinal environment, particularly peptide antibiotics, which are mostly active on gram positive bacteria, but also enzymes that show lytic activity against *P. aeruginosa*. The *B. clausii* has established to be extremely resistant to gastric acidity and most antibiotics (Marseglia *et al.*,2007). Also the studies can be concluded that the use of probiotics can be a solution to the antibiotic resistance problem to pathogens and can be compared with (Imperial & Ibana ,2016).

Increasing the resistance of *P. aeruginosa* against β -lactamase inhibitor antibiotics may be related to extreme β -lactamase construction and or activity of efflux mechanism may also add to the full expression of β -lactamase resistance in *P. aeruginosa* (Hoiby *et al.*,2010).

Table 3 Susceptibility test of some probiotics with some supporting substances against *Pseudomonas aeruginosa* comparing with some types of cephalosporin antibiotics.

Treatments	Inhibition zone \pm sd
<i>S.bouldarii</i>	14 \pm 3.6
<i>B.clausii</i>	16 \pm 2.6
A.A+S.bouldarii	18 \pm 6.5
C.A +S.bouldarii	19 \pm 6.08
A.A+B.clausii	20.3 \pm 1.5
C.A+B.clausii	23.5 \pm 0.8
cefibem	1.3 \pm 2.3
ceftriaxone	10.3 \pm 2.08
ceflaxin	0 \pm 0

CONCLUSION

The current study indicated that there are several types of bacteria that cause postoperative wound infection, important one was *Pseudomonas aeruginosa* which cannot be treated with classic antibiotics because they have developed many types of resistance. It is better to resort to alternative treatment methods, including the use of probiotics. So, we concluded the possibility of using probiotic extract with some supported substances as an anti-bacterial *Pseudomonas aeruginosa* isolated from surgical wound infection.

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