The Effect of Polysorbate 80 on Antibiotics’ Sensitivity

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Abstract
This study aims to assess the direct antibacterial effect of the surfactant polysorbate 80 on Pseudomonas aeruginosa isolate when it was tested alone, as well as to assess the combining effects of this surfactant with the commonly used antibiotics. Agar well diffusion method was carried out to assess the antibacterial activity of different concentrations of polysorbate 80 alone on the bacterial isolate. Antibiotic sensitivity test was performed by testing number of antibiotics applied against pseudomonal isolate, firstly; by applying the surfactant alone at different concentrations, secondly, without polysorbate 80 and thirdly; with the presence of various concentrations of the surfactant (0.025, 0.05, 0.1, 0.2, 0.4, 0.6, 1, 2, 3, 4, 5 and 6) % (v/v media) applied to the media. The results of the study revealed that polysorbate 80 alone had no antibacterial activity at the tested concentrations but it showed a highly potentiative effects when it was combined with some antibiotics. These effects appeared either in changing the bacterial sensitivity profile to antibiotics which was reflected by changing the resistance state to sensitivity state of Pseudomonas aeruginosa isolate toward cloxacillin, cephalothin, cefotaxime and gentamicin at (5) % (v/v) and (6) % (v/v). Or by significantly enhancing the pre-existing anti-pseudomonal activity which was reflected by increasing the diameter of inhibition zones produced by meropeneme (p<0.01) and amikacin (p<0.05) when tested against Pseudomonas aeruginosa.

As a conclusion, polysorbate 80 had no antibacterial activity but it had produced a potentiative effect with some antibiotics while it had no effects with the others.
**Introduction**

Surface-active agents (surfactant) are defined as amphiphilic or amphipathic molecules, *i.e.*, they have one part that has an affinity for non-polar media and one part that has an affinity for polar media, these molecules form oriented mono-layers at interfaces and show surface activity (1). Polysorbates are class of emulsifiers used in some pharmaceuticals and food preparations. They are often used in cosmetics to solublize essential oils into water-based products (6). They are perhaps one of the most commonly used nonionic surfactants, since they are sugar-based surfactant and considered as the safest surfactant (renewable surfactant) (7).

It has a hydrophilic- lipophilic balance (HLB) equal to (15) therefore it forms O/W emulsions (1).

It is proposed that polysorbate 80 alters the outer lipid structure of the envelope of certain fungi allowing easier access of polymyxin B to the underlying membrane and induction of rapid changes in pH, temperature or tonicity and thus enhance the killing activity. (14).

*Pseudomonas aeruginosa* is responsible for about 11% of nosocomial infections and the most common pathogens of urinary tract infections (19). *P. aeruginosa* possesses an intrinsic resistance to a variety of antimicrobial agents. This intrinsic resistance results from a low outer membrane permeability and expression of specific drug efflux pumps such as those coded by the *mexAB–oprM* operon (20,21). The tripartite pumps expressed from this operon couple the inner and outer membranes for extrusion of a range of antibiotics including tetracycline, chloramphenicol, quinolones, novobiocin, macrolides, trimethoprim, and β-lactams (21, 26). In addition, *P. aeruginosa* produces a chromosomally encoded group I, class C cephalosporinase designated AmpC (27). Although AmpC is produced at very low basal levels in wild-type strains, its expression is highly inducible in the presence of certain β-lactams antibiotics which are β-lactamase inducers such as cefoxitin or imipenem (28). In fact, the antipseudomonal penicillins (such as ticarcillin or piperacillin) and cephalosporins (such as ceftazidime or cefepime) are very weak AmpC inducers, despite the fact that they are hydrolyzed by this enzyme (28, 29). For this reason, during treatment with these weak inducers, mutants showing constitutively high level AmpC production (AmpC- derepressed mutants), leading to the failure of antimicrobial therapy (30).

This study aims to explore the possible interaction and the effects of different concentrations of polysorbate 80 on some of antibiotics activity against *Pseudomonas aeruginosa* isolates.

**Materials and methods**

Autoclave (National; Japan), incubator (Memmert; Germany), water bath (Memmert; Germany), micropipette (Oxford; USA), sensitive electric balance (A&D; Japan), polysorbate 80 (Tweem 80)(GCC; UK), Müller –Hinton agar (Hi media; India), antibiotics include: Amikacin (AK; 30 µg) Cloxacillin (CX; 1 µg), Ceftazidime (CTZ; 30 µg), Cefotaxime (CTX; 30 µg), Cefixime (CFZ; 5 µg), Doxycycline (DO; 30 µg), Gentamicine (GN; 10 µg), Cephalothine (KF; 30 µg), Cefepeme (CFP; 30 µg), Meropenem (MER; 10 µg), Ticarcilin (TIC; 75 µg) and Vancomycin (VA; 30 µg).

**Polysorbate 80 (Tweem ®80) solutions**

A series of dilutions (1, 2, 3, 4, 5, and 6) % (v/v media) were prepared from a stock solution (100% ps 80). Solutions with concentrations below 1% (0.025, 0.05, 0.1, 0.2, 0.4, and 0.6) % (v/v media) were prepared from a stock solution of 5% (v/v water) ps 80. All concentrations of ps 80 added to culture media specified to assess the effect of tested antibiotics efficiency in the presence of ps 80.
**Bacterial culture media:**

Müller-Hinton media was prepared according to manufacturer instructions. A weight of 38g of Müller-Hinton agar powder was dissolved in (1) liter distilled water with gentle heating to achieve complete solubilization and then it was sterilized by autoclave (autoclave was operate at $121^\circ$C, $15$ lb/ inch$^2$) for 15 minute. The media used for antibiotic sensitivity tests.

**Bacterial identifications:**

*P. aeruginosa* isolates was obtained from the Department of Microbiology - College of Medicine-University of Babylon. The isolate was identified and diagnosed according to Forbes, *et al.*, (31).

**Antibiotic sensitivity test:**

The test was carried out according to Kirby –Baur, *et al.*, (32) method by using a pure culture of previously identified bacteria. The inocula used in the test were prepared by adding growth of five isolated colonies grown on blood agar plate to 5mls of nutrient broth, then incubated at $37^\circ$C for 18 hours to produce a standardized bacterial suspension.

A. A sterile swab used to obtain inoculum from the standardized broth culture and the inoculum was then streaked on Müller-Hinton agar plates.

B. Antibiotics discs were placed on the surface of the medium at evenly spaced intervals with flame sterilized forceps.

C. Aerobic incubation of plates were done usually for an overnight with an optimal incubation time of 14 hours at $37^\circ$C.

D. The inhibition zones (in mm) were measured by using a ruler.

E. The resultant inhibition zones compared with the standard zones recommended by The Clinical Laboratory Standards Institute Documentations (33).

**The influence of polysorbate 80 on *Pseudomonas aeruginosa* bacterial isolate:**

The experiment was performed by applying agar well diffusion method as follows: (34)

A. Plates of Müller-Hinton were cultured with freshly prepared bacterial isolates of *P. aeruginosa*.

B. A sterile cork borer was used to make wells of 3mm in diameter in the agar plates with uniform distance.

C. A volume of 50µl of each ps 80 concentrations were added to the wells.

D. The plates were incubated for overnight at $37^\circ$C.

F. The inhibition zones were observed and measured by a ruler.

**The influence of antibiotics against bacterial isolates in presence of polysorbate 80 in the culture media:**

The solution of Müller-Hinton medium was used to dilute ps 80 stock solutions to a series of desired concentrations (0.025, 0.05, 0.1, 0.2, 0.4, 0.6, 1, 2, 3, 4, 5 and 6) % (v/v media). The resultant solutions were shaken well before and after autoclaving to ensure miscibility of mixed solutions then poured into Petri-dishes and left for complete solidification. This Müller-Hinton medium then became containing ps 80 with different concentrations.
Results

There was no antibacterial activity detected for ps 80 at different concentrations (0.025-6) % (v/v media) when tested alone against *P. aeruginosa* isolate.

The results of antibiotic sensitivity test alone on *P. aeruginosa* isolate were revealed that all antibiotics resisted by *P. aeruginosa* isolate except meropeneme (sensitive) and amikacin (intermediate sensitive).

The results of the combining effects of polysorbate 80 with the antibiotics revealed the following:

1. Cloxacillin was resisted by the bacterial isolate when polysorbate 80 present in the media in concentrations bellow 6% (v/v media) but with this concentration of polysorbate 80, the antibiotic cloxacillin gave a significant inhibitory effects against this bacterial isolate.

2. Cephalothin showed a significant inhibitory effect against bacterial isolates when polysorbate 80 added to the media at 5% (v/v media) and 6% (v/v media) concentrations only. However, Cephalothin resisted by bacteria when polysorbate 80 concentrations used bellow these concentrations.

3. Cefotaxime showed an intermediate inhibitory effect against bacterial isolates when polysorbate 80 added to the media at 5% (v/v media) and 6% (v/v media) concentrations only. However, bacterial isolate resisted cefotaxime when polysorbate 80 concentrations bellow these concentrations.

4. Polysorbate 80 showed a net highly significant ($p<0.01$) potentiative enhancement of the antibacterial activity of meropenem when added to the media, a maximum antibacterial activity observed at 6% (v/v media) polysorbate 80 as shown in figure (2).

5. There was a significant ($p<0.05$) net increase in the inhibitory effect produced by amikacin against the tested bacterial isolate when polysorbate 80 added to the media in different concentrations as shown in figure (3).

6. There was an observed resistance to gentamicin in spite of the presence of polysorbate 80 at different concentrations except at 5% and 6% (v/v media) for which an intermediate sensitivity was observed.

Discussion

Polysorbate 80 showed no antibacterial activity at its different concentrations (0.025-6% v/v media) alone against *P. aeruginosa isolates*. This result was in agreement with (14, 35, 36), although they used polysorbate 80 in concentrations up to 3% (v/v media).

For all other used antibiotics, in the absence of polysorbate 80, the bacterial isolate of *P. aeruginosa* exhibited only sensitivity toward meropenem. Such a result was in agreement with Voutsinas, et. al., (37). It also showed an intermediate sensitivity toward amikacin while a resistance behavior appeared for the others. This result was in agreement with Caselli, et. al., (38). Such a result was compatible to the fact that *P. aeruginosa* is considered as the most common naturally drug resistant bacteria (39, 40).

The potentiative effects of polysorbate 80 on antibiotics sensitivity explained as the following:

A. Cloxacillin, cephalothen and cefotaxime

The sensitivity of *P. aeruginosa* isolate to cloxacillin when polysorbate 80 added at 6% (v/v media), sensitivity to cephalothen when polysorbate 80 added at 5% and 6% (v/v media) concentrations and Cefotaxime when polysorbate 80 added at 5% and 6% (v/v media) can be explained by considering the following suggestions:
I. Cloxacillin and cephalothin are not popular antibiotics to be used as anti-pseudomonal antibiotics. Therefore, they exhibited inhibitory effects against the bacterial isolate when polysorbate 80 used at 6% (v/v media) for cloxacillin and at 5% , 6% (v/v media) for cephalothin. In fact, they are not listed by CLSI, 2007 as anti-pseudomonal antibiotics.

At the high concentrations of polysorbate 80, antibiotics with less anti-pseudomonal activity able to exert an inhibitory effect against bacterial isolate, probably due to their ability to interacted with polysorbate 80 either by complex formation or by solubilization within the micelles of the non-ionic agent giving them the ability to bypass bacterial barriers or to modify these barriers. This suggestion run in the same context of Brown and Winsley, (1971). Modification of bacterial barriers include alterations of porin proteins in the cell membrane causing reduced permeability and blocking entry of the drug as well as the use of an efflux mechanism to pump out the antibiotic as it crosses the membrane (41, 42).

II. Polysorbate 80 may affects the growth of bacteria when used at high concentrations. This result was in agreement with Matosic, et. al., (43), or potentiate the toxicity of antibiotics against bacteria by increase the aqueous solubility of antibiotics and thus increase their distribution to the media cause contamination of the bacterial environment (2). This suggestion was in agreement with Shin et. al., (16). Nevertheless, when the concentration of the added polysorbate 80 reaches 3% (v/v media), it showed no any antibacterial activity, a result homogenous with that introduced by Huot, et. al., (18).

B. Meropenem

The potentiative enhancement of the antibacterial activity of meropenem produced by polysorbate 80 when added to the media and the maximum antibacterial activity observed at 6% (v/v) ps 80.

Polysorbate 80 is among many drugs directly or indirectly influences cell membrane properties; as for example, the interactions between proteins and phospholipids or the formation of complexes between ligand molecules and phospholipids can lead to disruption of the membrane so that, it becomes highly permeable (44). Because of the unique structure of a lipid matrix consisting of phospholipids and embedded proteins, the interaction of surfactant molecules with polar head groups, non polar hydrocarbons, or both, can induce several changes in the membrane (15).

C. Aminoglycosides: (amikacin and gentamicin)

Two of the most important mechanisms that affect P. aeruginosa membrane are phase separation and domain formation. Phase separation was observed when mixtures of phospholipids incorporated into a biological membrane and when the individual components exhibit certain differences in their structure (15).

Accordingly, because ps 80 has a phospholipid-like function can induce phase separation and domain formation (the protrusion of the plasma membrane). This domain formation will affect membrane integrity (45,46) as well as the function of many membrane-linked enzymes, such as phospholipases (47, 48). This mechanism greatly enhances the efficacy of amikacin since the resistance to amikacin attributed to a reduction of its uptake owing to a reduced permeability and, as such, typically referred to as impermeability resistance (49),this mechanism weakened by ps 80. Because amikacin is not a substrate for a number of aminoglycoside-modifying enzymes, or which retain their antibacterial activity after modification, thus it used to treat infections due to gentamicin-resistant organisms, including MRSA (50).
Gentamicin is a charged amphiphilic drug. Its diffusion through Gram-negative bacterial cell walls may result in an even more complex type of drug–membrane interaction leading to a partial resistance (15). In addition, gentamicin resists by bacterial isolate due to production of inactivating enzyme. The inactivating enzymes overwhelmed the perturbation of plasma membrane that result from phase separation and domain formation. Therefore, it could suggest that inactivating enzyme consumed the passed gentamicin molecules. However, saturation of enzymes occurred at higher concentrations of polysorbate 80 due to maximum changes in conformation of plasma membrane, which lead to passage of large number of molecules thus intermediate sensitivity observed.

**Conclusions**

It is concluded that:
The present study concludes the following:

1. Ps 80 has no any antibacterial activity against *P. aeruginosa* isolate when this bacterial isolate was exposed to it without antibiotics.

2. Ps 80 at (5) and (6)% (v/v) rendered the resistant *P. aeruginosa* isolate sensitive to cloxacillin, cephalothin, cefotaxime and gentamicin where without ps 80 or with concentrations bellow than these concentrations this isolates was resistant to those antibiotics.

3. Ps 80 showed a highly potentiative enhancement of the antibacterial activity of meropenem when added to the media and a maximum antibacterial activity was observed at 6% (v/v) ps 80 against *P. aeruginosa*.

4. Ps 80 showed a potentiative enhancement of the antibacterial activity of amikacin against *P. aeruginosa*.

![Figure (1): Structure of polysorbate 80 (Lobback, et. al., 2007)](image-url)
Figure (2): Effect of different concentrations of polysorbate 80 on inhibition zone produced by meropenem when tested against *p. aeruginosa*

Figure (3): Effects of different concentrations of polysorbate 80 on antibacterial efficacy of amikacin when tested against *P. aeruginosa*
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