

# NUOVE COMPOSIZIONI PER IL TRATTAMENTO DELL'INFEZIONE DA *HELICOBACTER PYLORI*

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RESEARCH ARTICLE

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## Polysorbate 80 and *Helicobacter pylori*: a microbiological and ultrastructural study

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## **Conclusion on which is based the possibility that in a few years physicians might lack effective antibiotics to treat *H. pylori* infection**

- Chemoresistances are the main cause of therapeutic failure of *H. pylori* infection.
- The occurrence of acquired resistances in such species is very high, because of certain characteristics that *make H. pylori* hypermutable .
- Mutation rates in *H. pylori* are in fact 10–700 fold higher than that observed in other species, for instance *Escherichia coli*.
- The mechanisms of acquired chemoresistance in *H. pylori* include its significant genetic competence, i.e. the ability to recombine exogenous DNA

**Stress conditions, such as the antibiotic treatment and the exposure to HCl, induce numerous events in this species, which may end up enhancing the frequency of chemoresistances**

- a) The transcription and translation of natural competence genes, which increase the frequency of transformation;
- b) transcription of a lysozyme-like protein, which promotes DNA donation from the neighbouring cells;
- c) the stimulation of DNA uptake machinery, which increases the import of foreign DNA.
- d) An additional source of genetic exchange is the transfer of genomic islands by conjugative mechanisms;
- e) the antibiotics utilizable in the treatment of *H. pylori* infection are limited;
- f) it is mandatory to use them in combination of two or three at a time to be efficacious

# **Characteristics of polysorbate 80**

- ✓ **Polysorbate 80 is a nonionic surfactant used as an emulsifier in food, for example ice cream**
- ✓ **It is also used in bacterial broth cultures to prevent foam formation and as an excipient in numerous medications and vaccines against influenza to stabilize aqueous formulations.**
- ✓ **It is reputed to be a generally safe and well-tolerated compound.**

# **Antimicrobial characteristics of polysorbate 80**

- Polysorbate 80 (Tween 80) have been employed for their nature of surfactant to produc microemulsion systems, which caused a complete loss of viability of *Staphylococcus aureus* and *Escherichia coli*.
- Other surfactants, such as dodecyl-maltoside and octyl-glucoside, enhanced the effectiveness of antibiotics used in the treatment of human pulmonary tuberculosis for their permeabilizing properties
- Finally, some researchers examined some substances, included Tween detergents, considered, in the past, efficacious treatments for peptic ulcer, and found that they were able to inhibit H. pylori receptor binding in vitro.

# **Antimicrobial activity of polysorbate 80 against *H. pylori* (22 strains) and prevalence of chemoresistances**

- MBCs of polysorbate 80 ranged from 2.6 (1.1) to 32 (0) µg/mL
- MBC<sub>50</sub> was 16 µg/mL; MIC<sub>90</sub> was 32 µg/mL

<b>Antibiotics</b>	<b>% of resistant strans</b>
<b>Amoxicillin</b>	<b>0</b>
<b>Clarithromycin</b>	<b>23.9</b>
<b>Metronidazole</b>	<b>36.3</b>
<b>Levofloxacin</b>	<b>4.5</b>
<b>Tetracycline</b>	<b>9.0</b>

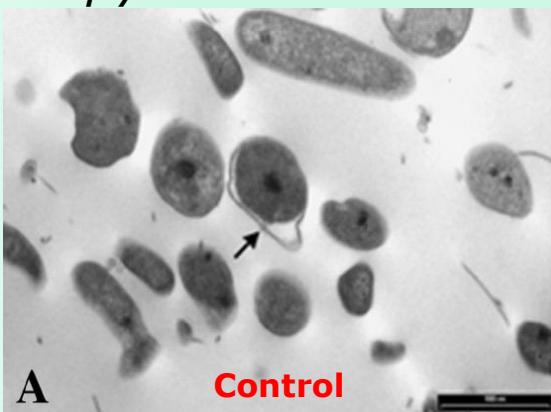
## **Polysorbate 80 shows a synergistic antimicrobial activity**

- When used in association, the MBCs of polysorbate 80 decreased by 2–4 times and those of clarithromycin and metronidazole by 2–16 times, compared to the respective MBCs of drugs used alone. The effect of the association of polysorbate 80 with amoxicillin, or levofloxacin, or tetracycline was negligible
- Two strains were highly resistant to clarithromycin, with MBCs of 320 µg/mL and 2500 µg/mL. In the presence of polysorbate 80, clarithromycin's MBCs decreased by 16 times and 1000 times, respectively, i.e. to 20 µg/mL and 2.5 µg/mL, which still are in the range of resistant values (threshold = 1 µg/mL).

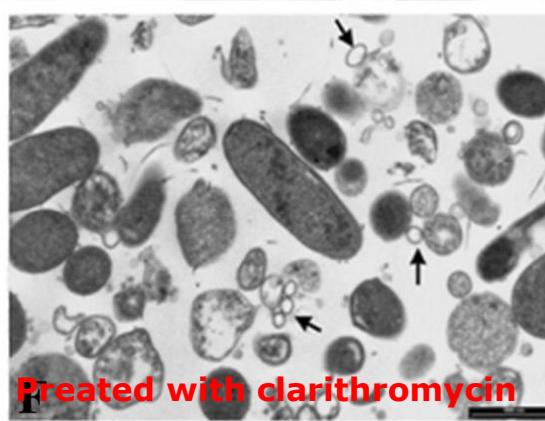
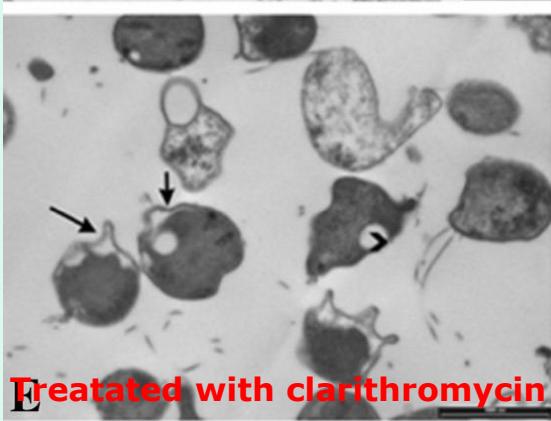
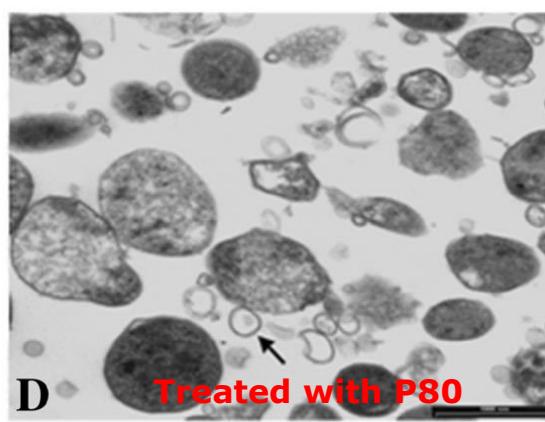
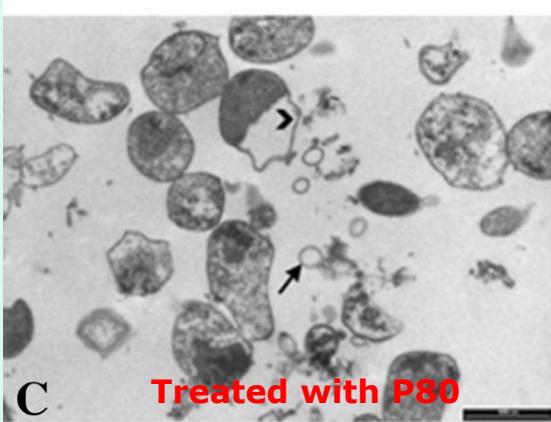
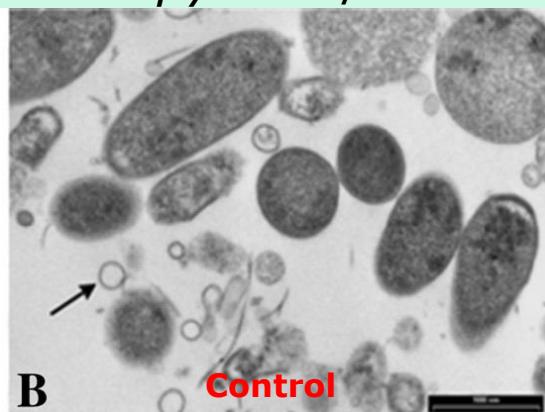
## **Ultrastructural anomalies caused by polysorbate 80 upon *H. pylori* strains**

- alterations of the bacterial shape,**
- swelling of the organisms,**
- loss of the normal and homogeneous cytoplasmic structures,**
- anomalies in the bacterial envelope especially in the outer membrane**
- presence of numerous vesicles**

*H. pylori* CCUG 17874



*H. pylori* C/M-R2

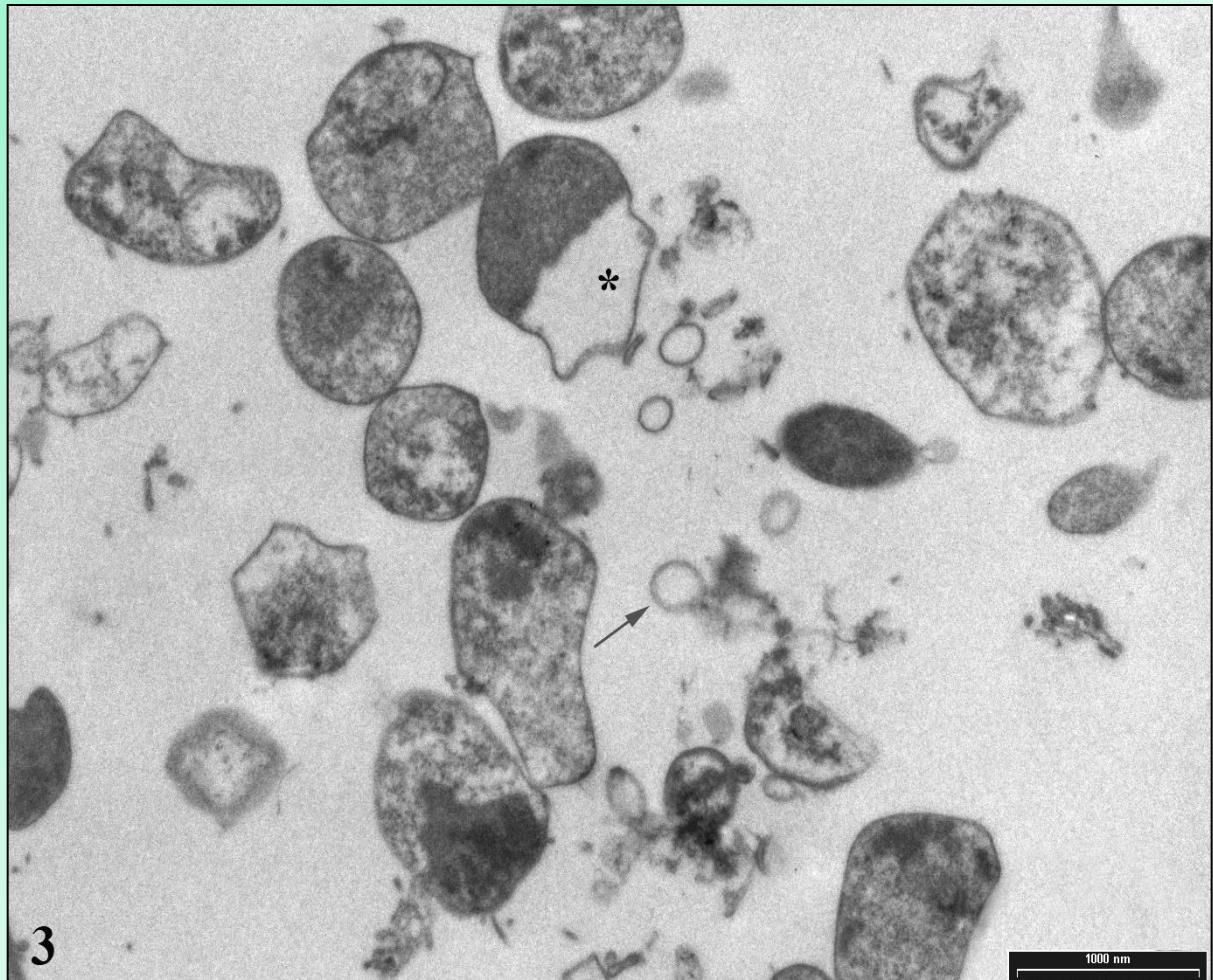


- Alterations of the bacterial shape
- Swelling of the organisms
- Loss of the normal and homogeneous cytoplasmic structures
- Anomalies in the bacterial envelope especially in the outer membrane
- Presence of numerous vesicles

**Micrografia al TEM del  
ceppo di *H. pylori* CCUG  
17874 non trattato.**  
**L'esame  
ultrastrutturale mostra  
forme piuttosto  
regolari con citoplasma  
omogeneo e rari  
scollamenti della  
parete/membrane dal  
citoplasma (freccia).**



**Microografie al TEM  
del ceppo di *H.  
pylori* CCUG 17874  
trattato con  
polisorbato 80. I  
batteri sono alterati  
di forma e rigonfi; il  
citoplasma perde la  
sua struttura  
omogenea e  
compatta. Sono  
presenti vescicole  
(freccia). In figura 3  
un batterio mostra il  
distacco della  
parete/membrane  
dal citoplasma  
(asterisco).**



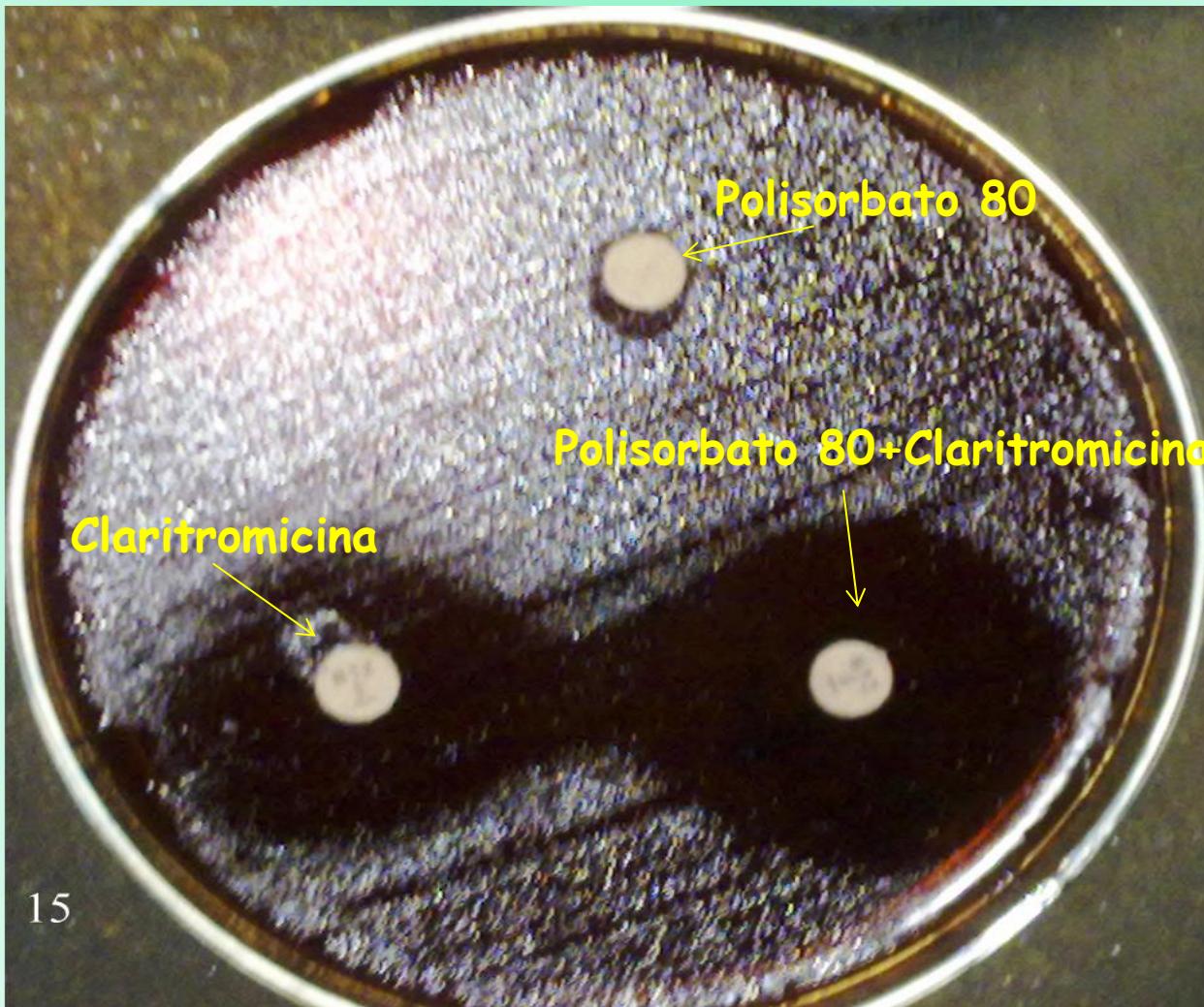
**Micrografia al TEM  
del ceppo di *H.  
pylori* M/C-R2  
trattato con  
metronidazolo. I  
batteri non  
presentano  
alterazioni  
morfologiche.**



**Micrografia al TEM  
del ceppo di *H.  
pylori* M/C-R2  
trattato con  
polisorbato 80 e  
metronidazolo.  
I batteri sono  
alterati di forma,  
rigonfi con  
citoplasma  
granulare.  
Segnaliamo la  
presenza di  
vescicole (frecce)**



# Effetto sinergico dell'associazione polisorbato 80/claritromicina



- Attività nei confronti del *H. pylori* ceppo 328:

I'attività del metronidazolo associato al polisorbato aumenta di 64 volte e quella della claritromicina associata al polisorbato di 8 volte.

- Associazione di polisorbato, metronidazolo e claritromicina nei confronti del ceppo resistente M/C-R1:

I livelli di resistenza al metronidazolo e alla claritromicina si sono ridotti rispettivamente di 10 e di 16 volte.

- Associazione di polisorbato e claritromicina nei confronti del ceppo resistente M/C-R2:

in questo caso, la CMB si è ridotta fino a livelli di claritromicina considerati pressoché inattivi (1 µg/ml)

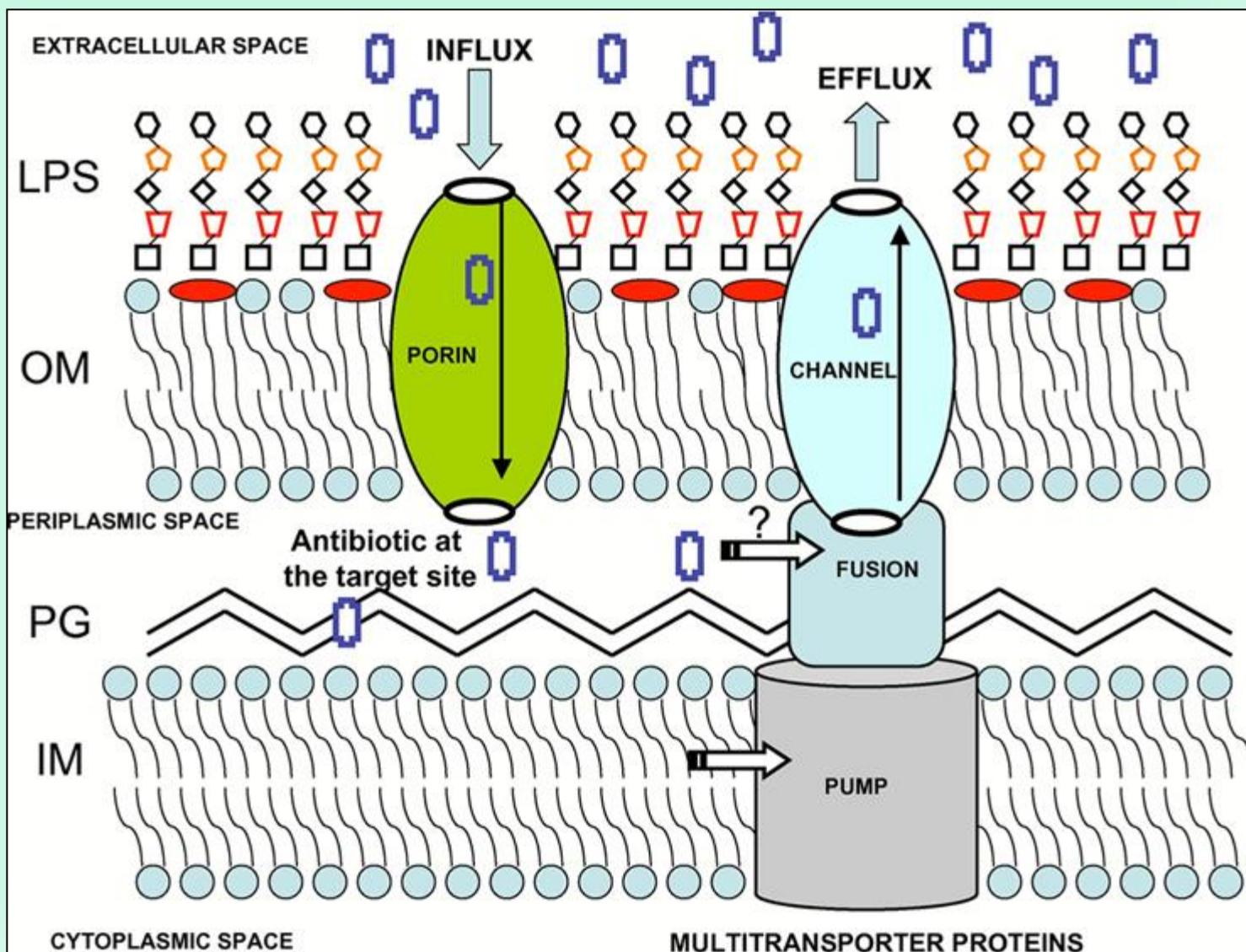
## **Attività antimicrobica del polisorbato 80**

- **Polisorbato 80 incrementa la permeabilità di membrana favorendo l'ingresso e aumentando la concentrazione degli antibiotici nello spazio periplasmatico dei batteri.**
- **Le alterazioni morfologiche osservate sono verosimilmente da attribuire all'azione di tipo detergente del polisorbato 80. Infatti, ogni volta che i batteri sono stati trattati con questo detergente, sono state osservate tipiche anomalie ultrastrutturali quali: alterazione della forma e rigonfiamento dei batteri, perdita della struttura omogenea e compatta del citoplasma , presenza di vescicole....”**

# **Hypothesis on the antimicrobial activity of polysorbate 80**

- Two strains were highly resistant to clarithromycin, with MBCs of 320 µg/mL and 2500 µg/mL
- In the presence of polysorbate 80, clarithromycin's MBCs decreased by 16 times and 1000 times, respectively, i.e. to 20 µg/mL and 2.5 µg/mL, which still are in the range of resistant values (threshold = 1 µg/mL)

# Pattern of efflux proteins in gran-negative bacteria



# Pattern of efflux proteins in gran-negative bacteria

