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Outer membrane vesicles (OMVs) as antiobiotic resistance carriers

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INTRODUCTION:

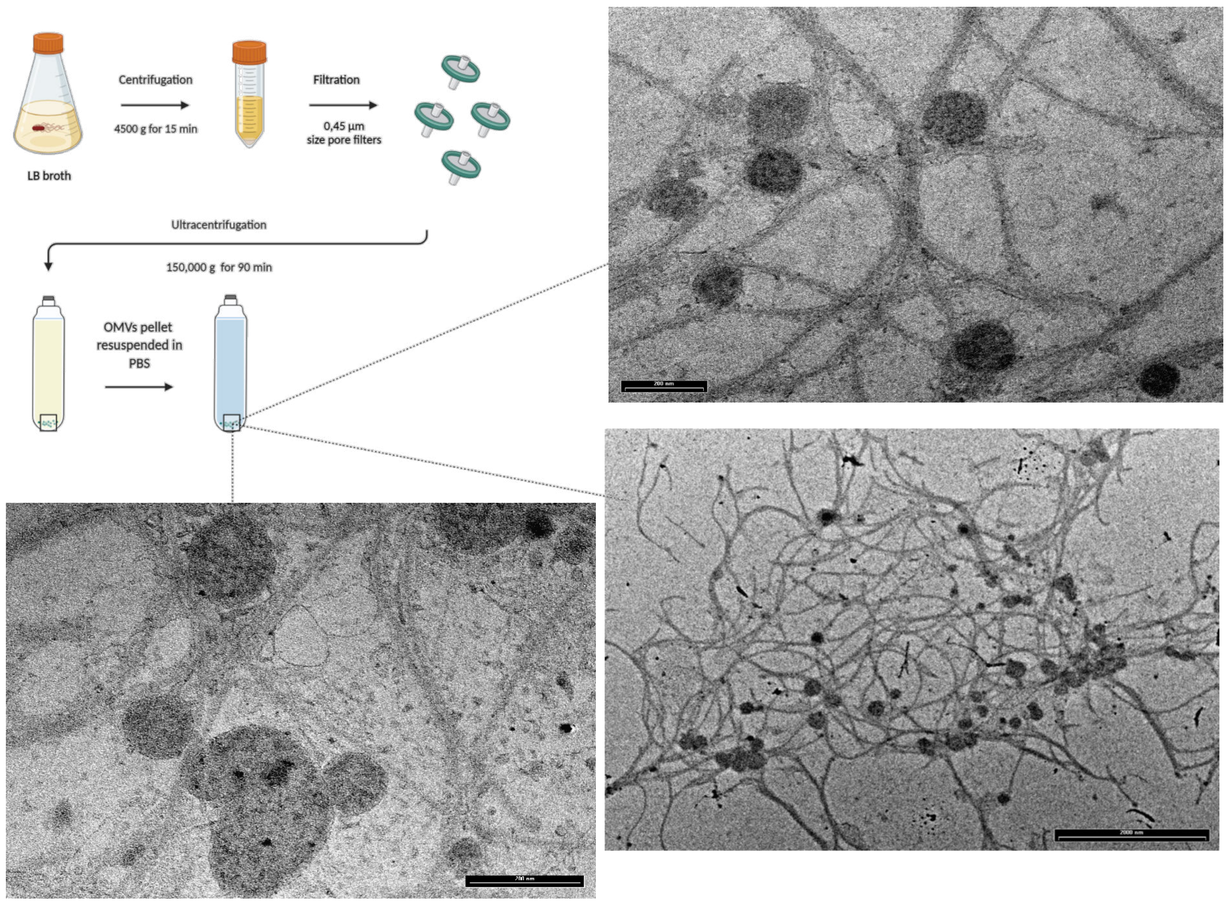
Most bacteria release membrane vesicles (MVs) with sizes ranging from 20 to 400 nm in diameter. These MVs contain different cargo molecules and have specific functions that influence different biological processes, including virulence, horizontal gene transfer, export of cellular metabolites, phage infection and cell-to-cell communication. MVs were first found to be produced in Gram-negative bacteria, therefore they are often referred as Outer Membrane Vesicles (OMVs) [1]. OMVs are small, spherically bilayered blebs, released into extracellular milieu from the outer membrane of Gram-negative bacteria [2].

AIM OF THE STUDY:

Since no studies have been conducted on Salmonella Infantis' OMVs yet and on their role in antibiotic resistance, a preliminary study has been carried out on this topic. The aim of the study was to phenotypically evaluate antimicrobial susceptibility of several strains of S. Infantis isolated from broiler chickens and humans, to collect the OMVs and to quantify the amount of β-lactamase enzyme in both the bacterial strains and the OMVs. To assess the antimicrobial susceptibility and the presence of ESBL, S. Infantis isolates were analysed by disk diffusion test and by microdilution method.

MATERIALS AND METHODS:

OMVs isolation was performed on one β-lactam *Salmonella* Infantis resistant strain. *Salmonella* was grown on Luria-Bertani broth until the late exponential phase. β-lactamase enzyme was quantified by Nitrocefin test.



Electron micrograph of IP8 derived OMVs. The image depicts vesicles mixed with residual pili and flagella (TEM, scale bar 2000 nm).

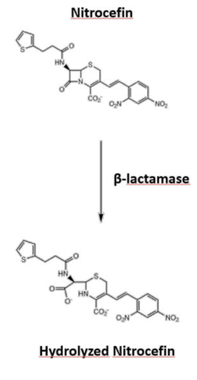
Transmission electron microscopy was used to investigate OMVs morphology. Proteins both from cell lysate and from OMVs were quantified by Bradford assay and separated by SDS-PAGE. Statistical analysis was applied to calculate the β-lactamase activity value, expressed as milliunit per milligram (mU/mg).

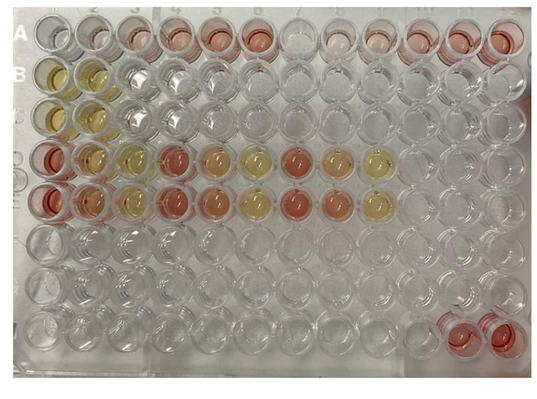
CONCLUSION:

Further studies should be carried out to characterize OMVs' content, in order to understand how OMVs can regulate and serve as a vehicle of β-lactam resistance in bacteria.

Nitrocefin test:

the assay is based on the hydrolysis of Nitrocefin, a chromogenic cephalosporin giving rise to a coloured product that can be measured with a spectrophotometer (OD_{490}).



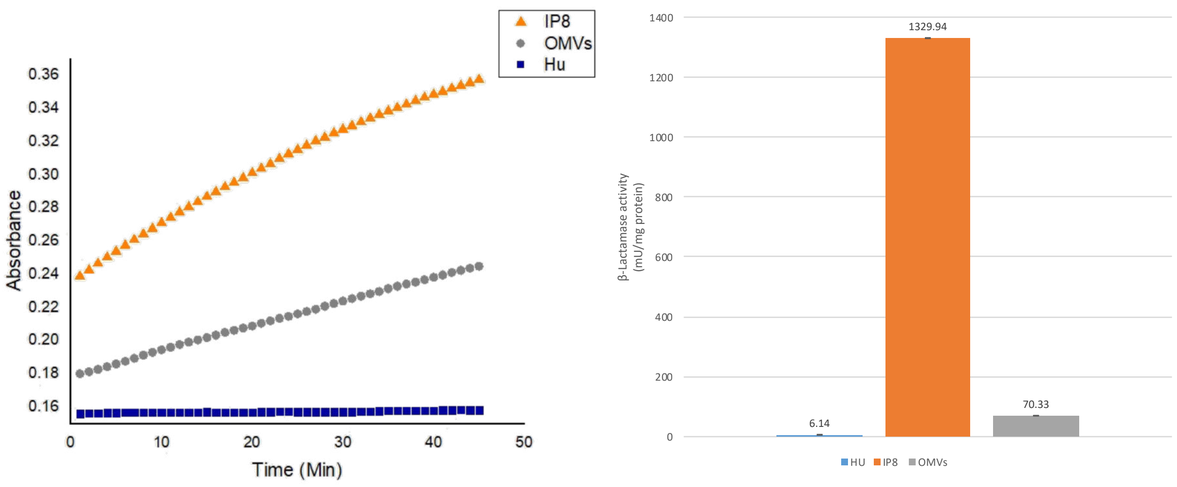


RESULTS:

Our results demonstrated the presence of β-lactamase activity in OMVs isolated from *S*. Infantis resistant strain and confirmed that also in *Salmonella* Infantis the enzyme gets packaged in OMVs from bacterial periplasm during vesicles biogenesis.

Antibiotics	Strains					
	IP8		F		Human	
	MIC		MIC		MIC	
Cefotaxime (FOT)	>4	R	>4	R	<0.25	S
Ceftazidime (TAZ)	2	S	2	S	<0,05	S
Ampicillin (AMP)	>64	R	>64	R	<1	S
Meropenem (MERO)	<0,03	S	<0,03	S	0,03	S
Nalidixic acid (NAL)	128	R	128	R	<4	S
Ciprofloxacin (CIP)	0,25	R	0,06	S	<0,015	S
Sulfamethoxazole (SXT)	>1024	R	>1024	R	32	S
Trimethoprim (TMP)	>32	R	>32	R	<0,25	S
Tetracycline (TET)	64	R	64	R	<2	S
Azithromycin (AZI)	8	S	8	S	4	S
Chloramphenicol (CHL)	<8	S	64	R	<8	S
Tigecycline (TGC)	<0,25	S	0,25	S	<0,25	S
Colistin (COL)	<1	S	<1	S	<1	S
Gentamycin (GEN)	<0,5	S	<0,5	S	<0,5	S

Antimicrobials susceptibility of *S*. Infantis isolates by both dilution test.



Nitrocefin test: (*left*) enzyme kinetic of β-lactamase in bacterial crude lysate (IP8 and Hu) and in the isolates outer membrane vesicles (OMVs); (*Right*) Relative β-lactamase activity expressed per milligram of protein.

REFERENCES

[1] Toyofuku M, Nomura N, Eberl L: Types and origins of bacterial membrane vesicles. Nature Reviews, 2019. [2] Mac Donald, Ian and Kuehn M: Offense and defense: microbial membrane vesicles play both ways. Res Microbiology, 2012.