THÉRAPIE COMBINÉE NANOMÉTRIQUE CONTRE LA SALMONELLA ENTERICA SER. TYPHIMURIUM CHEZ LES PORCINS

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CDPQ Centre de développement du porc du Québec inc.

30 ans 1992.2022

INTRODUCTION

La salmonelle

↓

Salmonellose

↓

Infectieuse zoonotique

crampes abdominales | diarrhées | vomissements | fièvre

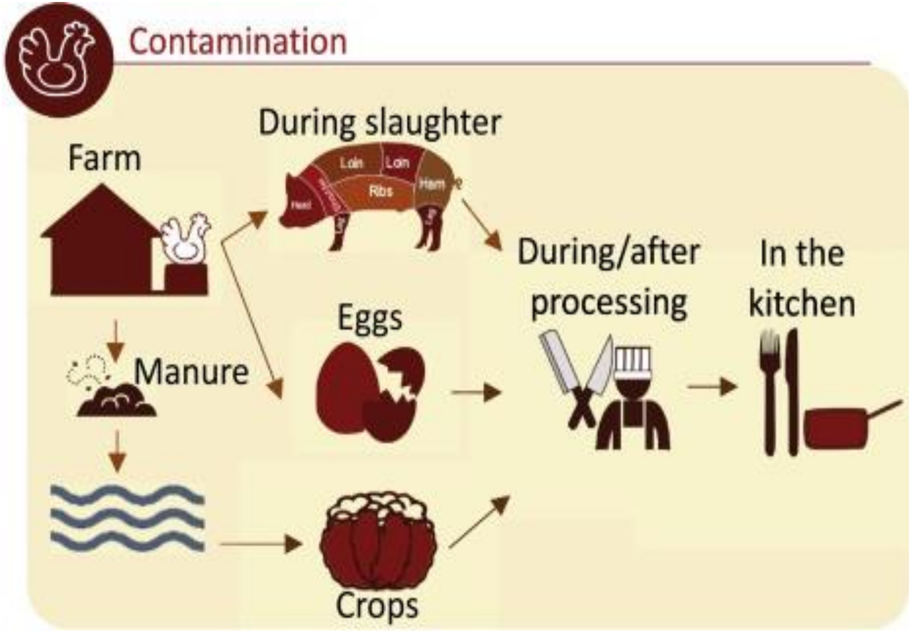

Fig 1. Route de contamination (Staes et al., 2019)

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OBJECTIF

La réponse immunitaire innée à S. Typhimurium repose essentiellement sur les cellules phagocytaires.

Objectif : Concevoir et tester un nanosystème qui cible spécifiquement S. Typhimurium en laissant les micro-organismes non pathogène résidant dans l'intestin du porc.

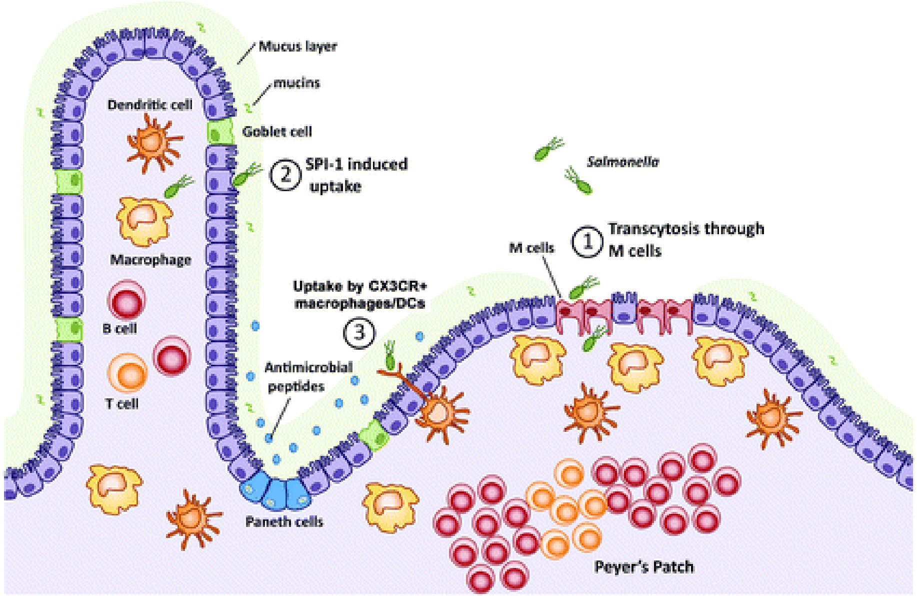

Figure 2. Broz et al., 2012

LES ÉTUDES PRÉSENTES

METHODES

Sensibilité antimicrobienne aux antibiotiques commerciaux

- ❖ Diffusion sur disque de Kirby-Bauer
- ❖ Concentration minimale inhibitrice
- ❖ Tableau de damier- Test de synergie antimicrobiens

Séquençage du génome entier

- ❖ MEGARes : Base de données antimicrobienne pour le séquençage à haut débit

Pathogénicité intracellulaire

- ❖ Études de survie intracellulaire - IPEC-J2

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RESULTATS

SÉQUENÇAGE DU GÉNOME ENTIER DU *S.* TYPHIMURIUM ET ANALYSE ANTIMICROBIENNE

Salmonella Typhimurium multirésistant	
Gène	Résistance
aac(6'), kdpE, aac3, aph(4), antd(3'), aphd(3'), aph(6)	Aminoglycosides
BacA	Bacitracine
Pbp2, ampH	Bêta-lactames
ble	Glycopeptides
HNS, sdiA, msbA	Multirésistance aux médicaments
ermD, CpxAR, yogI, marR, marA, BCR, MdtK, CRP, emrB, emrA, emrR, acrA, acrb	Résistance aux médicaments et aux biocides
pmrG, soxS,	Résistance aux médicaments et aux métaux
robA, arcD, BaeR, BaeS, gesA, gesB, gesC, MdtC, MdtB, MdtA	Résistance aux médicaments, aux biocides et aux métaux
floR	Phénicol
sulII, sulIII	Sulfonamides
tetM, tetA	Tétracycline
dfrA	Triméthoprime

Table 1. Whole genome sequencing of Salmonella Typhimurim isolate

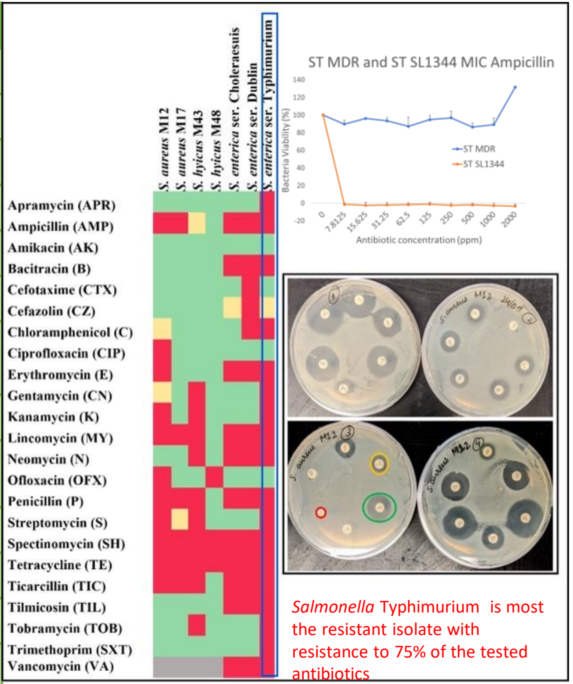

Figure 3. Antimicrobial susceptibility of tested bacterial isolates

EFFICACITÉ DE NeACT CONTRE LES RÉSIDUS INTRACELLULAIRES DE SALMONELLA TYPHIMURIUM (Caco-2)

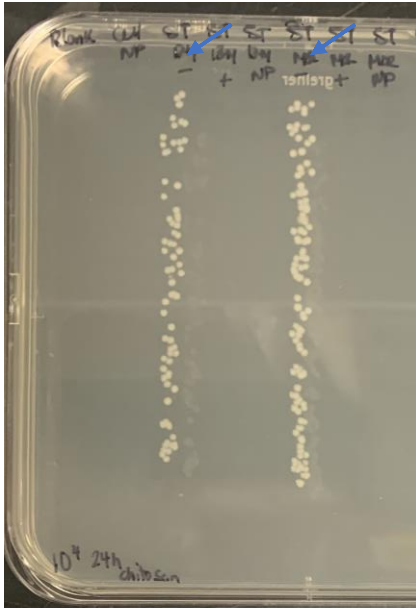

Figure 4. Hanging drop culture of intracellular S. Typhimurium in caco-2 cells treated with chitosan nanocomposite

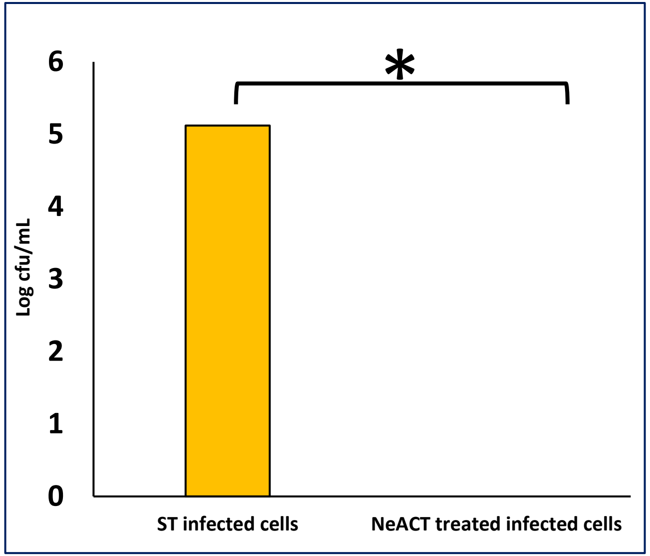

Figure 5a. Efficiency of the chitosan nanocomposite to combat intracellular pathogens.
The nanocomposite at 12.5 µg/mL reduced intracellular infection of *Salmonella* Typhimurium to the Caco-2 cells by >5 log cfu/mL.

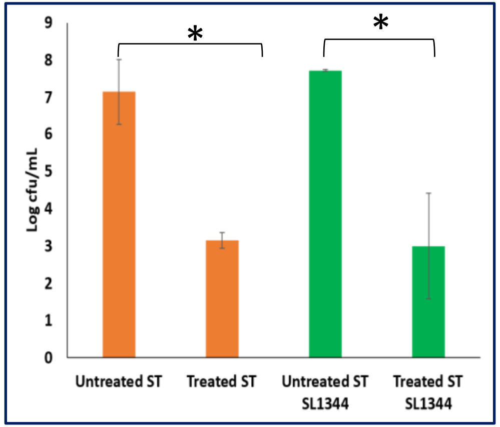

Figure 5b. Efficiency of the silver nanocomposite to combat intracellular pathogens.
The nanocomposite at 62.5 µg/mL significantly (p>0.5) reduced intracellular infection of *Salmonella* Typhimurium and *Salmonella* Typhimurium SL1344 to the Caco-2 cells by 2.26 and 2.5 folds.

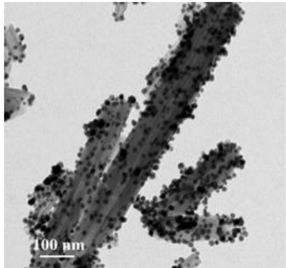

Figure 6. Transmission electron microscopy image GH-TA-Ag-NT particle . (Majumder et al., 2022)

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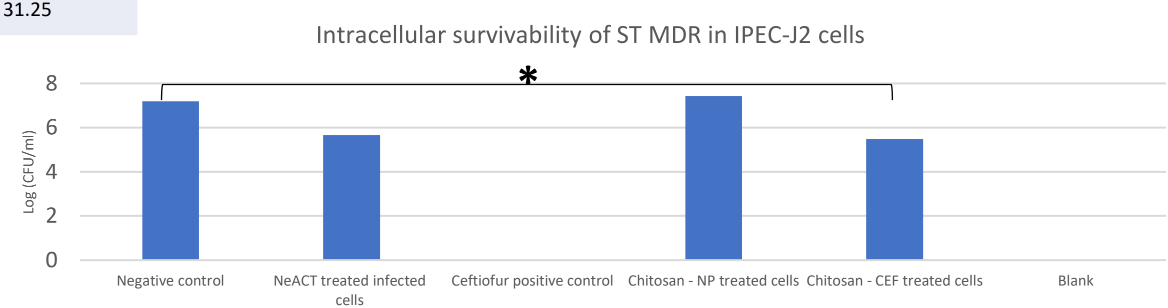
Drug	Conc. (ppm)
NeACT	12.5
CF	15.62
CH-NP	250
CH-CF	31.25

EFFICACITÉ DE NeACT CONTRE LES RÉSIDUS INTRACELLULAIRES DE SALMONELLA TYPHIMURIUM (IPEC-J2)



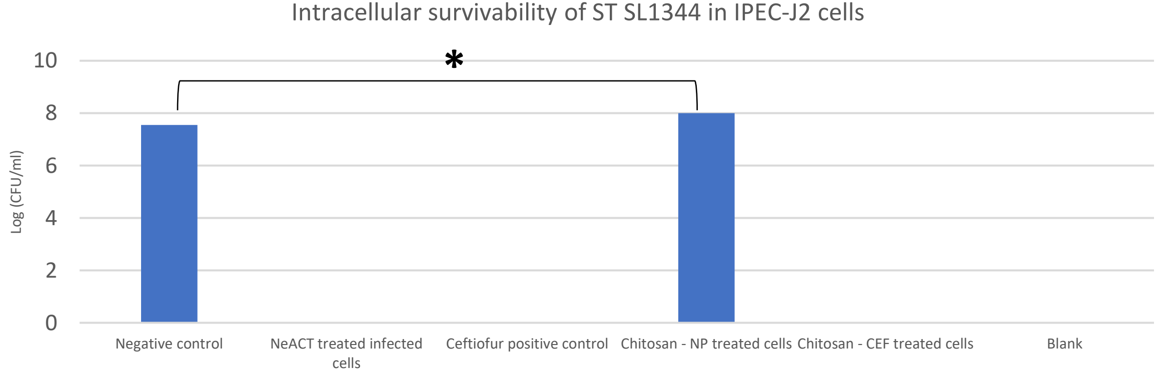
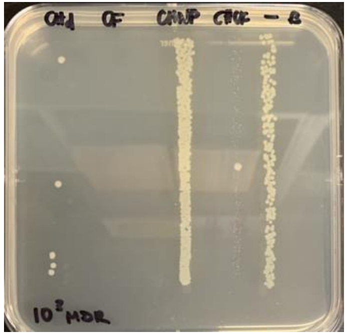


Figure 7. Efficiency of the chitosan nanocomposite to combat intracellular S. Typhimurium in IPEC-J2 cells

A 12.5 ug/mL of the NeACT successfully inhibited intracellular residing ST MDR by >1.5 log cfu/mL.



A 12.5 ug/mL of the NeACT successfully inhibited intracellular residing ST SL1344 by <7 log cfu/mL.

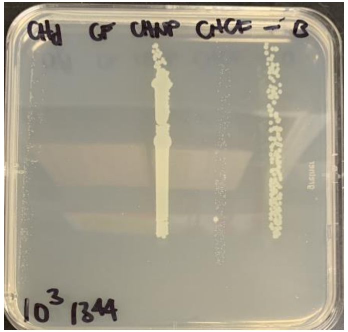


Figure 8. Hanging drop culture of intracellular S. Typhimurium in IPEC-J2 cells treated with chitosan nanocomposite

ÉTUDES FUTURES

DÉVELOPPEMENT DU NANOSYSTÈME

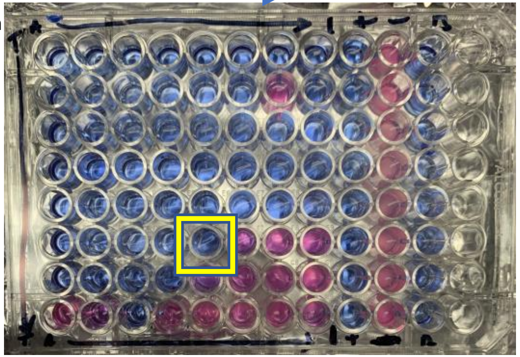


Figure 9. Checkerboard assay against Salmonella Typhimurium

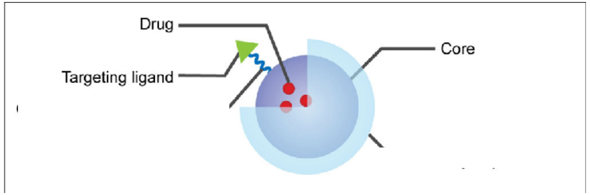
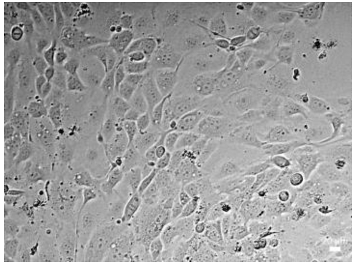
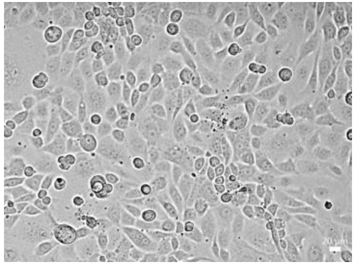


Figure 11. Drug loaded nanoparticle schematic (Li et al., 2020)

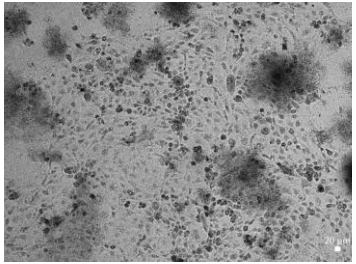
1. Isolation des vacuoles
2. Développement et analyse de nanoparticules



IPEC-J2 cells (2x10[4]) before infection



IPEC-J2 cells treated with 31.25ug/mL (Amoxicillin)& 15.625ug/ml (Tazobactam)



IPEC-J2 cells treated with 31.25ppm Amoxicillin

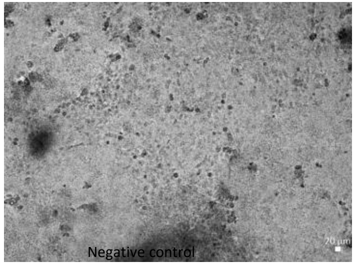


Figure 10. Combination Therapy Intracellular Data in IPEC-J2 Cells

RÉFERENCE

Broz, P., Ohlson, M. B., & Monack, D. M. (2012). Innate immune response to salmonella typhimurium, a model enteric pathogen. Gut Microbes, 3(2), 62–70. https://doi.org/10.4161/gmic.19141

Collado-Romero, M., Arce, C., Ramírez-Boo, M., Carvajal, A., & Garrido, J. J. (2010). Quantitative analysis of the immune response upon Salmonella typhimurium infection along the porcine intestinal gut. *Veterinary research*, *41*(2), 23. https://doi.org/10.1051/vetres/200907

Henry, A., Letellier, A., Côté, J. C., Desmarais, G., Lachapelle, V., Bergeron, N., ... & Fravalo, P. (2019). Salmonella contamination in a network of 10 pig farms interconnected within the same cooperative. *Veterinary Record Open*, *6*(1), e000269.

Figure 1. Staes, I., Passaris, I., Cambré, A., & Aertsen, A. (2019). Population heterogeneity tactics as driving force in salmonella virulence and survival. *Food Research International (Ottawa, Ont.)*, *125*, 108560–108560. https://doi.org/10.1016/j.foodres.2019.108560

Figure 2. Broz, P., Ohlson, M. B., & Monack, D. M. (2012). Innate immune response to salmonella typhimurium, a model enteric pathogen. Gut Microbes, 3(2), 62–70. https://doi.org/10.4161/gmic.19141

Figure 6. Majumder, S., Viau, C., Brar, A., Xia, J., & George, S. (2022). Silver nanoparticles grafted onto tannic acid-modified halloysite clay eliminated multidrug-resistant Salmonella Typhimurium in a Caenorhabditis elegans model of intestinal infection. *Applied Clay Science*, *228*, 106569.

Figure 11. Li, M., Zhao, G., Su, W. K., & Shuai, Q. (2020). Enzyme-responsive nanoparticles for anti-tumor drug delivery. *Frontiers in Chemistry*, *8*, 647.

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