Inhibition of gentamicin-resistant pathogenic E. coli and salmonella

The use of antibiotics in poultry has been under scrutiny for more than two decades. The European ban of antibiotic growth promoters (AGP) was decided in 1996, with enforcement beginning 10 years later at the beginning of 2006. This action led to an overuse of antibiotics for therapeutic purposes in most of the countries affected by the ban.

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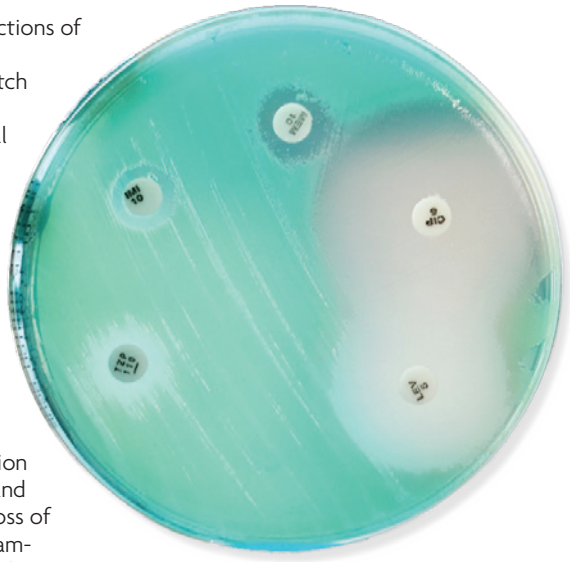
The application of antimicrobials for therapy, prophylaxis, and growth promotion in the production of broiler chickens has been associated with the emergence and proliferation of antimicrobial-resistant enteric bacteria.

Although human exposure to antimicrobial-resistant bacteria through food has been examined extensively, little attention has been paid to occupational and environmental pathways of exposure.

Among the different antibiotics used in the production of poultry, gentamicin is reported to be the most widely used aminoglycoside in several regions of the world.

In accordance with geographic acceptance, gentamicin has been administered via subcutaneous injection to day-old chicks in an effort to prevent and treat infections of E. coli and salmonella.

Keeping poultry healthy from hatch to market is key in modern farm management. Prevention of clinical diseases is much more effective and cost-efficient than treating diseases once they manifest themselves.



Inappropriate use of antibiotics leads to an increase in microbial resistance

Despite early successes in prevention of disease, the use of gentamicin and other antimicrobials has led to a loss of sensitivity of commonly found Gram-negative bacteria such as E. coli and Salmonella spp.

In hospitals, there have been explosive outbreaks of nosocomial infection in man caused by gentamicin-resistant bacteria of many species.

Bacillus strains can inhibit pathogens

Some bacilli are particularly strong in the production and secretion of antimicrobial peptides that act against potentially pathogenic organisms.

Certain highly selected strains of bacilli from Chr. Hansen have the ability to produce an array of these type of compounds, like fengycin, iturin, mycosubtilin, and surfactin that have the capacity to inhibit both Gram-negative and Gram-positive pathogens.

In general, antimicrobial peptides are cationic (positively charged) and display hydrophobic or amphophilic properties and, in most cases, their activity is directed against the membranes of other bacteria.

Several models of the mechanisms of action of these cationic peptides have been proposed.

The main actions identified and described involve the formation of channels through which ions and water can pass and/or the disruption of bacterial cytoplasmic membranes; these actions have a lethal effect on the targeted bacteria.

Three principal steps are required for the manifestation of these antimicrobial effects:

- Binding of peptides to the bacterial membrane.
- Aggregation of the peptides within the membrane.
- Formation of channels.

For Gram-negative bacteria, antimicrobial peptides must cross the negatively charged outer wall which contains lipopoly-saccharides (LPS). For Gram-positive bacteria, antimicrobial peptides must pass through the outer cell wall which contains acidic polysaccharides.

These types of antimicrobial peptides were proven to be produced and secreted

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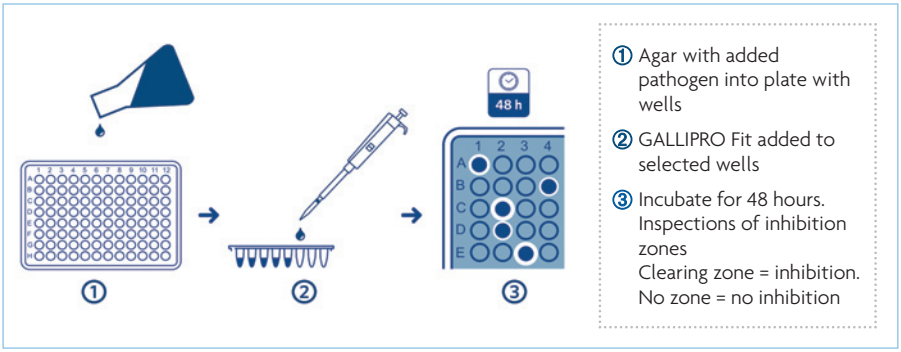
Fig. 1. Agar plate diffusion methodology.



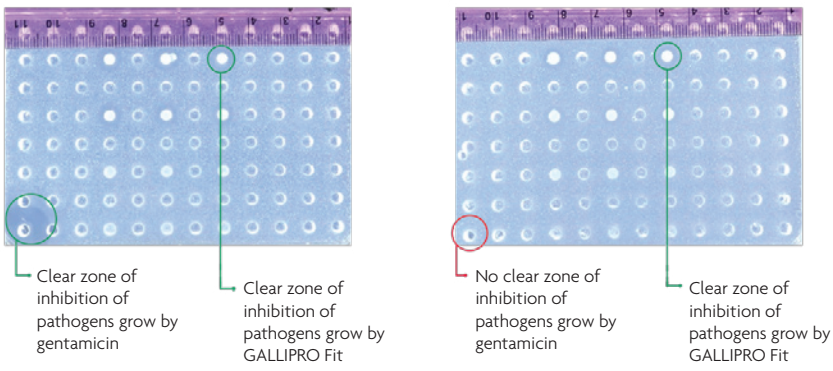


Fig. 2. Examples of gentamicin sensitive or resistant isolates.

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by bacillus-based probiotics from Chr. Hansen. GALLIPRO Fit is a combination of three strains of two species of bacilli that were selected for their capacity to inhibit Salmonella spp., E. coli, and Clostridium spp.

Retrospective review of the analysis of field-sourced pathogens from US poultry operations

In February 2020, the Pathogen Inhibition Assay tool was launched in North America. The singular purpose of this tool was to validate the capacity of GALLIPRO Fit to inhibit pathogens commonly found within commercial operations in the US. Field isolates of E. coli and Salmonella spp. are submitted by producers to a third-party laboratory, Southern Poultry Research Group (SPRG, Nicholson, GA).

Upon arrival, the inhibitory actions of GALLIPRO Fit against those pathogens are assessed.

During a one-year period, 48 samples of pathogenic E. coli and five samples of pathogenic salmonella were isolated from various production sites across different types of birds (Table 1) and from various sources from the birds (Table 2).

Many samples were identified by the producers as 'unknown' to preserve confidentiality.

Pathogen inhibition assay

Upon arrival of the isolates to SPRG, the pathogens were mixed with selective agar and poured into a microtiter plate. A set of 96 wells were created in each plate using a pronged device designed for that purpose. GALLIPRO Fit was added to a subset of wells within the plate (see Fig. 1).

After incubation for 24 and 48 hours, the magnitude of the zones of inhibition around the wells that contained the probiotic were measured using a standard scoring system (Chr. Hansen SOP-07695).

As a putative positive control, gentamicin is added to the lowest left well.

For some samples of pathogens, growth was inhibited around the gentamicin-infused well, indicative of the inhibitory action of that common antibiotic on sensitive pathogenic organisms.

However, for other samples, there was a notable absence of inhibition. These pathogen were then assumed to be resistant to the actions of gentamicin (see Fig. 2).

The magnitude of inhibition obtained with GALLIPRO Fit is illustrated in Fig. 3.

Table 1. Host of the different isolated pathogens.

	E. coli	Salmonella
Broiler	14	
Broiler breeder	17	
Egg layer	4	
Turkey	13	
Unknown		5

	E. coli	Salmonella
Cloacal	22	
Intestine	4	
Liver	1	
Unknown	21	5

Table 2. Location of pathogen isolate harvest.

Inhibition of growth of pathogenic E. coli

These third-party in vitro results demonstrate the potential of GALLIPRO Fit to inhibit the growth of a wide spectrum of strains of E. coli. These isolates were collected in field conditions throughout the United States and were submitted by producers. Additionally, all of the major poultry species are represented, at various ages, and from a range of geographies.

- GALLIPRO Fit inhibited the growth of 47 of 48 field isolates of E. coli and five out of five field isolates of Salmonella spp.
- Of the 48 isolates of E. coli, 24 were observed to be resistant to the gentamicin used in the control well. All of the gentamicin-resistant strains were sensitive to GALLIPRO Fit.
- Of the five isolates of salmonella, one was observed to be resistant to the gentamicin used in the control. That isolate was sensitive to GALLIPRO Fit.

The Chr. Hansen Pathogen Inhibition Assay is an excellent tool to illustrate the potential of GALLIPRO Fit to significantly and meaningfully contribute to the pathogen mitigation programmes in commercial conditions.

It is notable that the gentamicin-resistant isolates of E. coli and salmonella were sensitive to the triple strain bacillus-based probiotic GALLIPRO Fit. It is very likely that the modes of action of these bacilli and their production and secretion of lipopeptide is different and most likely multifactorial compared to the singular action of gentamicin.

Consequently, the fight against gentamicin-resistant strains could be improved by a broader use of probiotics, such as GALLIPRO Fit, in poultry. ■

References are available from the authors on request

Fig. 3. Pathogen inhibition assay results for E. coli and salmonella by inhibition scores.

GALLIPRO Fit inhibition score	E. coli	Salmonella
0	1	0
+	5	0
++	29	5
+++	14	0
Interpretation	All but one were sensitive to GALLIPRO Fit. All the 24 gentamicin-resistant strains were sensitive to GALLIPRO Fit.	All were sensitive to GALLIPRO Fit. The gentamicin-resistant isolate was sensitive to GALLIPRO Fit.