



Impact of Micro-Aid® on the Bacterium *Salmonella*¹

Introduction

Bacteria belonging to the genus *Salmonella* are rod-shaped (bacillus), gram-negative, facultative anaerobic bacteria in the family *Enterobacteriaceae*. Based on their phenotypic profile, the genus *Salmonella* is divided into two species, *S. enterica* and *S. bongori*. *Salmonella enterica* is further divided into six subspecies that include over 2,500 serotypes. The majority (1,531) of these serotypes belong to *Salmonella enterica* subsp. *enterica* (I) and were originally given names such as Typhimurium, Dublin, and Infantis. *Salmonella enterica* subspecies *enterica* (I) is the only subspecies that has been associated with disease in warm-blooded animals and humans. It is estimated that 99.5% of *Salmonella* strains isolated from humans and warm-blooded animals belong to this subspecies. This gives rise to a food safety issue for animal agriculture industries.

Materials and Methods

Six serotypes of *Salmonella* were tested for their susceptibility to Micro-Aid® using the agar dilution test as described in the Clinical and Laboratory Standards Institute (CLSI) M31 document. The six different serotypes of *Salmonella* were *Salmonella* serotype (ser) Infantis, *Salmonella* ser Branderup, *Salmonella* ser Paratyphi, *Salmonella* ser Worthington, *Salmonella* ser Give and *Salmonella* ser Dublin. The minimum concentration of Micro-Aid® (MIC) required to inhibit the growth of each serotype was determined. This testing procedure has consistently provided quantitative results when Micro-Aid® has been tested against a variety of different bacterial isolates, both Gram-positive and Gram-negative, aerobic and anaerobic.

Following establishment of the MIC, a concentration-dependent killing assay (time-kill assay) was conducted against each serotype. The time-kill assays involved exposing broth cultures of each of the six *Salmonella* serotypes to Micro-Aid® concentrations that were 0, 0.5x, 1x, 2x, and 4x the MIC for each strain tested. Bacterial growth was monitored at hourly intervals over the six-hour time-course of the study. From data generated from the time-kill assays the impact Micro-Aid had on bacterial growth was assessed.

Results

All *Salmonella* serotypes tested were inhibited by Micro-Aid® concentrations of 10 mg (MIC) of saponin per mL of media (Table 1). These MICs are comparable to the MICs for many other species of bacteria, e.g., *E. coli*, *Clostridia perfringens*, etc., that have been tested to date.

Figure 1 depicts the time-kill assay results for *Salmonella* Worthington and the effect exposure to Micro-Aid® at increasing concentrations had on bacterial growth over a six-hour incubation period. The

conditions under which each growth curve was generated was the same, i.e., medium, inoculum, incubation temperature and time. The only difference was the presence of Micro-Aid® at concentrations of 5 mg/ml (0.5x MIC)

Table 1. Minimum Inhibitory Concentration (MIC) of *Salmonella* Bacteria Exposed to Micro-Aid®

Species	MIC (mg of Saponin/mL)
<i>Salmonella</i> Infantis	10
<i>Salmonella</i> Branderup	10
<i>Salmonella</i> Paratyphi	10
<i>Salmonella</i> Worthington	10
<i>Salmonella</i> Give	10
<i>Salmonella</i> Dublin	10

¹ This research was conducted by Dr. R. D. Walker of Anti-infectives Research Consultants (ARC), LLC.



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RESEARCH SUMMARY

10 mg/ml (1.0x MIC), 20 (2x MIC) and 50 mg/ml (4x MIC). Although only the results for *Salmonella* Worthington are presented, there were consistent similarities in results among all six *Salmonella* serotypes tested: **1)** The increase in viable cell counts in each control suspension (no Micro-Aid®); **2)** the patterns of growth of those suspensions exposed to Micro-Aid® at 0.5x their MICs were like the Controls; **3)** the suspensions incubated with Micro-Aid® at 1x their MICs exhibited a bacteriostatic growth pattern; **4)** growth pattern of those bacterial suspensions incubated with Micro-Aid® at concentrations that were 2x their MICs were mixed. Some appeared static whereas others died out during the six-hour incubation time; and **5)** the elimination of viable bacteria in all suspensions containing Micro-Aid® at 4x the MICs of the bacteria in that environment.

To assess the impact Micro-Aid® had on the growth of this bacterium in vitro we compared the percent increase in bacterial numbers for each Micro-Aid® concentration the bacterium was exposed to. While the results among all six serotypes was similar we present here the data for *S. Worthington* (Table 2). For the Control suspension, there was a 59.02% increase in viable cell numbers during the four-hour interval between the sample collected at 1 hour and the sample collected at 5 hours of incubation. For the bacteria incubated with Micro-Aid® at 0.5x the MIC, there was 51.53% increase in viable cell numbers. The cell count was further reduced to a 4.51% increase for those exposed to Micro-Aid® at 1x the MIC. For the bacteria incubated with Micro-Aid® at 2x and 4x the MIC, there were no viable bacteria recovered after a four-hour and two-hour exposure, respectively.

This data clearly shows that Micro-Aid® has an adverse effect on susceptible bacteria. It is not clear if this is due to Micro-Aid®'s impact on the growth rate of the bacteria or if Micro-Aid® is bactericidal to susceptible bacteria.

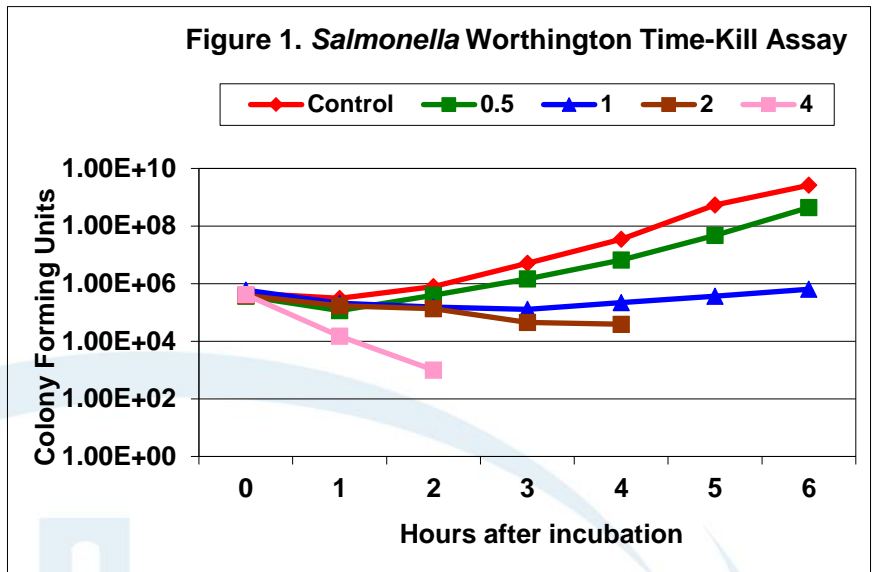


Table 2. Percent Increase in *Salmonella* Worthington Viable Cell Count When Incubated with Micro-Aid®

	Control	0.5x MIC	1x MIC	2x MIC	4x MIC
Concentration 1 hr	5.491	5.063	5.322	5.230	4.176
Concentration 5 hr	8.732	7.671	5.562	0.000	0.000
Difference	3.241	2.609	0.240	-5.230	-4.176
% Change 1 vs. 5 hr	59.02	51.53	4.51	--	--

Key Summary Points

- ❖ *Salmonella* were all found to have a similar minimum inhibitory concentration to Micro-Aid®.
- ❖ Although there are over 2,500 different *Salmonella* serotypes, the impact Micro-Aid® had on these isolates and the consistency of their response of this dataset can translate to *Salmonella* from other sources.
- ❖ Interestingly, this genus of bacteria was comparable in susceptibility to Micro-Aid® as all other members of the *Enterobacteriaceae* (e.g., *E. coli*) that have been tested to date.



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