

## Research Article

# FTIR Spectroscopic Study of Inhibition of Chloroxylenol-Based Disinfectant Against *Salmonella enterica* serovar Thyphimurium Biofilm

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### ABSTRACT

The present work was performed to determine the impacts of commercial disinfectants against biomass, viability, and biochemical composition of *Salmonella enterica* serovar Thyphimurium ATCC14028 biofilm. *Salmonella* Thyphimurium biofilm grown in microplates was exposed to commercial disinfectants namely sodium hypochlorite, benzalkonium chloride, chloroxylenol, and sodium dodecyl-benzene sulfonate-based disinfectants. Biofilm biomass, biofilm viability, and biochemical composition of the biofilm were determined using crystal violet assay, resazurin assay and Fourier transform infrared (FTIR) spectroscopy respectively. Results demonstrated that, among four commercial disinfectants, chloroxylenol-based disinfectant showed the highest inhibition against *S. Thyphimurium* biofilm. It remarkably hindered biofilm biomass and biofilm viability at all tested concentrations (0.78%-25%). Half-maximal biofilm inhibitory concentration ( $BIC_{50}$ ) of chloroxylenol-based disinfectant (5.06%) was found to be the lowest among the tested disinfectants. Meanwhile, *S. Thyphimurium* biofilm treated with chloroxylenol-based disinfectant exhibited changes in FTIR spectral peaks associated with lipid ( $1460\text{ cm}^{-1}$ ), protein ( $630\text{ cm}^{-1}$ ,  $702\text{ cm}^{-1}$ ,  $1550\text{ cm}^{-1}$  &  $1650\text{ cm}^{-1}$ ), and nucleic acid ( $1080\text{ cm}^{-1}$  &  $1229\text{ cm}^{-1}$ ). The findings of the present study suggest that the inhibition of chloroxylenol-based disinfectant against *S. Thyphimurium* biofilm is mediated by structural changes of biofilm.

**Key words:** Biofilm, chloroxylenol, disinfectant, FTIR spectroscopy, *Salmonella* Thyphimurium

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## INTRODUCTION

*Salmonella enterica* is a Gram-negative bacterium, rod-shaped, non-capsulated, facultatively anaerobic, and nonsporulating bacteria that belongs to the family Enterobacteriaceae. It is commonly found in water, soil, and animal feces. It is also a foodborne pathogen that becomes the most common cause of food-borne bacterial infection (Eng *et al.*, 2015). Salmonellosis is a disease caused by typhoidal and nontyphoidal *Salmonella* serovars that mainly cause food poisoning in the 20th century. Its infection has become a major public health issue in the United States, with an estimated 1.4 million incidents of illness and 600 deaths per year (Roth *et al.*, 2018). Recently, Nor *et al.* (2023) reported that *Salmonella* represents the most prevalent causative agent of gastroenteritis in Klang Valley, Malaysia. Over the last few decades, many works have been carried out to control food poisoning and other microbial infections caused by *Salmonella*.

Most bacteria can form biofilms including *Salmonella*. *Salmonella* biofilms can adhere to surfaces such as stainless steel, polyester, plastic, and aluminum (Alves *et al.*, 2015; Merino *et al.*, 2019; Othman & Yahya 2019; Tassinari *et al.*, 2019). Biofilms are a group of microbial cells that are adhered to a living or inert surface and encased themselves in a self-produced extracellular polymeric matrix (Mahat *et al.*, 2012; Yaacob *et al.*, 2021). Biofilm formation starts with bacterial attachment, followed by microcolony formation, biofilm maturity, and finally biofilm dispersion (Johari *et al.*, 2020; Jean-Pierre *et al.*, 2023). Biofilms are present everywhere such as in restrooms, hotels, the food industry, labs, and hospitals (Garrett *et al.*, 2008). They contribute to resistance to drugs, chemicals, physical stress, and the host immune system. Potential biofilm control measures include the use of antibiotics, antifungals, and natural products (Zawawi *et al.*, 2020; Yaacob *et al.*, 2021; Kamaruzzaman *et al.*, 2022a).

Since microbial infection caused by *Salmonella* has become a serious problem for public health, researchers have been trying to find a way to eradicate biofilm or at least control the growth of biofilm. Due to that, the use of disinfectants in cleaning procedures has become more crucial. Disinfectants are chemical germicides formulated to eliminate pathogenic bacteria on the surface. Unlike antibiotics, disinfectants have a broad spectrum of action against bacteria (Meyer & Cookson, 2010). The effects of disinfectants against bacterial biofilms have previously been investigated (Fouladynezhad *et al.*, 2013; Kart *et al.*, 2014; Lineback *et al.*, 2018; Capita *et al.*, 2019). The microorganisms' ability to survive is somehow related to the formation and existence of biofilm on the surfaces (Bressler *et al.*, 2009). Cells that are surrounded by the biofilm matrix showed phenotypic differences from planktonic cells that may contribute resistance to disinfectants (Egual *et al.*, 2014).

Biofilm formation is a dynamic event in bacteria. Understanding how disinfectants can control biofilm growth is a fundamental step to creating effective control measures. Fourier transforms infrared (FTIR) spectroscopy is a vibrational spectroscopic technique for detecting molecular changes in a membrane by identifying functional groups in the membrane as well as molecular bonds between chemical compounds. FTIR spectroscopy techniques have become widely studied by researchers to determine its application in biological study especially in foodborne pathogens (Neu & Lawrence, 2010; Duygu *et al.*, 2012; Mohamed *et al.*, 2017). There have been few studies being carried out using FTIR spectroscopy related to *Salmonella* species (Amamcharla *et al.*, 2010; Preisner *et al.*, 2010; Campos *et al.*, 2018). With FTIR spectroscopy, cellular macromolecules including protein, lipids, carbohydrates, and nucleic acid can be identified through a spectrum produced from specific infrared radiation (IR) absorption in the range between 4000 and 600  $\text{cm}^{-1}$ . Information regarding carbohydrates, proteins, and nucleic acid in the FTIR spectra provides insights into biofilm structure (Ariafar *et al.*, 2019). To date, the efficacy of commercially available disinfectants against *S. Typhimurium* biofilm and FTIR spectral changes remain not well studied. Therefore, the present work was performed to determine the impacts of selected commercial disinfectants namely sodium hypochlorite, benzalkonium chloride, chloroxylenol, and sodium dodecyl-benzene sulfonate-based disinfectants against *S. Typhimurium* biofilm.

## MATERIALS AND METHODS

### Chemicals

The following chemicals were used herein: The lists of chemicals used in this study were nutrient broth (Difco Laboratories, USA), ethanol (Merck, Germany), phosphate buffer saline (PBS) (Sigma, USA), sodium chloride (NaCl) (Sigma, USA), crystal violet stains (Sigma, USA), resazurin (Sigma, USA), dimethyl sulfoxide (DMSO) (Merck, Germany), sodium hydroxide (NaOH) (Sigma, USA), ethylenediaminetetraacetic acid (EDTA) (Sigma, USA), sodium dodecyl sulfate (SDS) (Sigma, USA), phenylmethylsulfonyl fluoride (PMSF) (Sigma, USA).

### Microorganism

*Salmonella enterica* serovar Thyphimurium ATCC14028 obtained from Microbiology Laboratory, Faculty of Applied Sciences, UiTM Shah Alam was grown at 37 °C in nutrient broth. Culture purity was regularly confirmed by Gram staining and biochemical test. The bacterial inoculum was adjusted to an optical density (OD) of 0.7 at 600 nm before crystal violet and resazurin assays.

### Disinfectants

Commercial disinfectants used in this study are shown in Table 1. They were tested in the range between 0.78% (v/v) and 25% (v/v).

**Table 1.** Active ingredients contained in commercial disinfectants

Type of disinfectants	Active ingredient
Disinfectant A	Chloroxylenol
Disinfectant B	Sodium dodecyl benzene sulfonate
Disinfectant C	Benzalkonium chloride
Disinfectant D	Sodium hypochlorite

### Crystal violet assay

The effect of *S. Typhimurium* biofilm biomass following exposure to commercial disinfectants was evaluated in a 96-wells microplate (Kamaruzzaman *et al.*, 2022b). Overnight inoculum (150  $\mu\text{L}$ ) and test solution (50  $\mu\text{L}$ ) were added to the microplate wells. An equal volume of fresh broth and intellectual property (IP)- protected antibiofilm cocktail were also added as negative and positive controls, respectively. The microplates were incubated overnight at 37 °C for 24 h. After discarding the medium, the biofilm fractions were rinsed with distilled water twice, heat-fixed at 60 °C for 30 min, stained with 0.5% (w/v) Crystal violet for five min, de-stained with sterile distilled water thrice and let to dry at room temperature, solubilized with 200  $\mu\text{L}$  of 95% (v/v) ethanol for 10 min and measured at 600 nm using ThermoFisher Scientific microplate reader.

### Resazurin assay

The viability of *S. Typhimurium* biofilm after to exposure commercial disinfectants was also evaluated in a 96-wells microplate (Kamaruzzaman *et al.*, 2022b). A stock of 0.02% (w/v) resazurin was prepared and stored at 4 °C in the dark. Overnight inoculum (200 µL) and test solutions (50 µL) were added to the microplate wells. An equal volume of fresh broth and intellectual property (IP)-the protected antibiofilm cocktail was also added as negative and positive controls, respectively. The microplates were incubated overnight at 37 °C. After 24 hr incubation at 37 °C, the medium was discarded whilst the biofilm fractions were rinsed with distilled water twice and heat-fixed at 60 °C for 30 min. The biofilm fractions were suspended in 170 µL of phosphate-buffered saline and 30 µL of 0.02% (w/v) resazurin was added to the wells. The microplate was incubated for 24 h at 37 °C and analyzed using microplate ThermoFisher Scientific microplate reader for measuring absorbance at 570 nm.

### Microplate biofilm assay for FTIR spectroscopy

*Salmonella* Typhimurium biofilm was grown in a 6-well microplate. Overnight inoculum (4 mL) was added to the microplate wells. Then, a volume of 1 mL of fresh nutrient medium was added. The microplate was incubated overnight at 37 °C. After 24 h period at 37 °C incubation, the content of the microplate was discarded while the microplate wells were rinsed with distilled water twice and the biofilm fraction was scrapped from the wall of the well after being suspended with 0.9% (w/v) sodium chloride (NaCl), 1mM phenylmethylsulfonyl fluoride (PMSF) and 1% (w/v) sodium dodecyl sulfate (SDS). The suspension then was transferred into 1.5 mL centrifuge tubes and vortexed for 3 min. Then, they were centrifuged at 4000 rpm for 15 min at 4 °C to obtain the pellet. The resulting pellets were dried in the oven at 60 °C for 2 h.

### FTIR spectroscopy

The biochemical composition of biofilm was determined using Perkin Elmer Spectrum One FTIR spectrometer. The dried cell pellets were positioned in direct contact with the diamond crystal, scanned in a range between 3000 cm<sup>-1</sup> and 600 cm<sup>-1</sup> with 4 cm<sup>-1</sup> spectral resolution, and ratioed against a background spectrum previously collected from the clean sampling surface. Spectral data analysis, visualization, and processing were performed by using Perkin Elmer Applications Spectrum software.

### Statistical analysis

Experimental data generated from Crystal violet and Resazurin assays were expressed as mean ± standard deviation with *n*=3. A significant difference between control and test groups (*p*<0.05) and strength of association were determined using an independent T-test and Pearson correlation coefficient test, respectively. The half-maximum biofilm inhibitory concentration (BIC<sub>50</sub>) values for the inhibition study of *S. Typhimurium* biofilm were determined by fitting biofilm biomass data using linear regression.

## RESULTS

### Inhibitory action of commercial disinfectants against biomass of *S. Typhimurium* biofilm

Figure 1 shows the effects of commercial disinfectants on the biomass of *S. Typhimurium*. Those disinfectants effectively inhibited the biomass of *S. Typhimurium* biofilm. The chloroxylenol-based disinfectant was found to show the highest inhibition against *S. Typhimurium* biofilm whereby at all test concentrations (0.78%-25%), it significantly (*p*<0.05) inhibited the biomass of *S. Typhimurium* biofilm.

### Inhibitory action of commercial disinfectants against the viability of *S. Typhimurium* biofilm

Figure 2 shows the effects of commercial disinfectants on the viability of *S. Typhimurium* biofilm. Those disinfectants also effectually impeded the viability of *S. Typhimurium* biofilm. All test concentrations of sodium hypochlorite and chloroxylenol-based disinfectants (0.78%-25%) significantly inhibited the viability of *S. Typhimurium* biofilm.

### Correlation between biomass and viability of *S. Typhimurium* biofilm

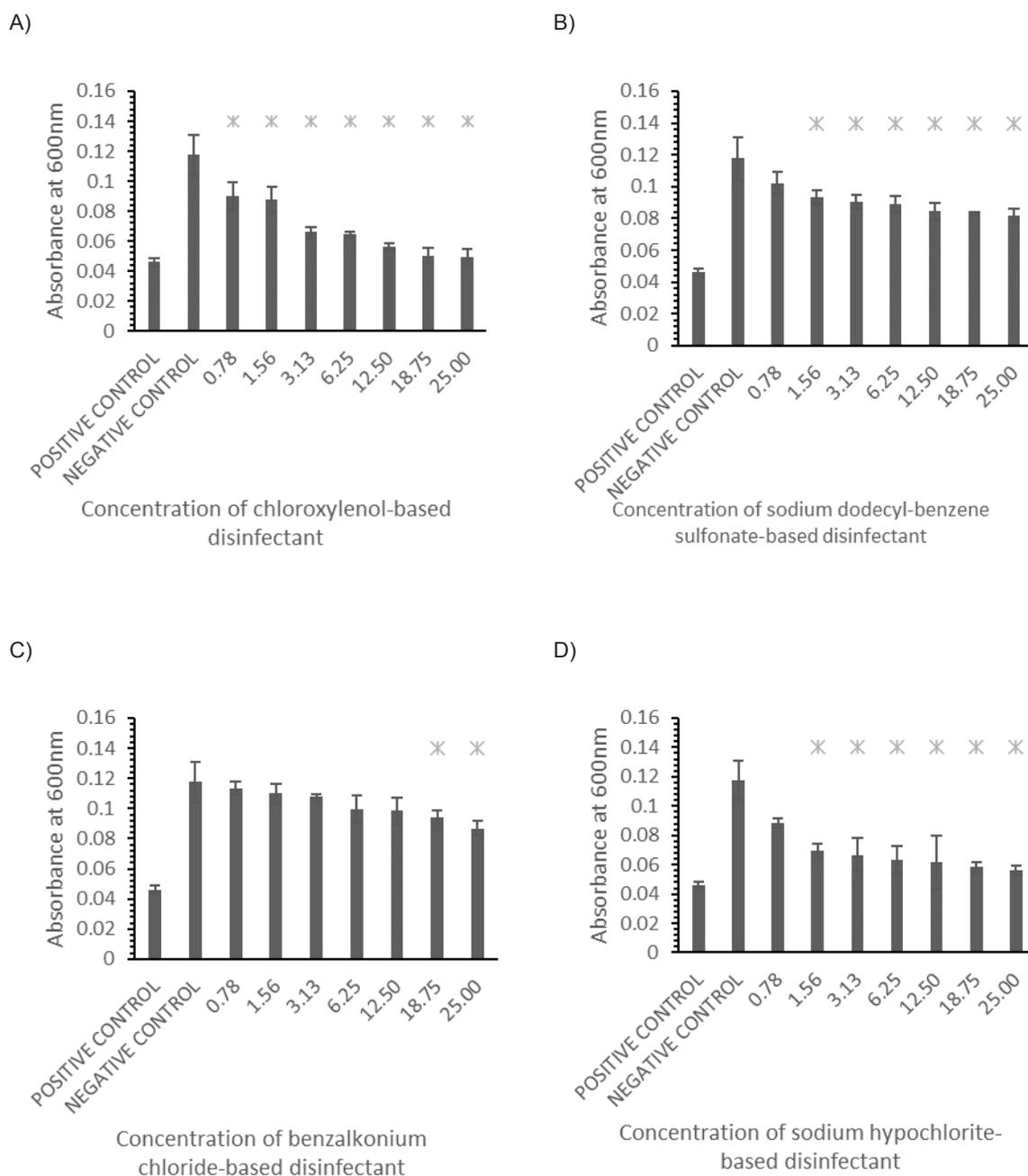
Figure 3 shows the strength of the association between biomass and viability of biofilm treated with commercial disinfectants. All the correlations values (sodium hypochlorite - 0.801; sodium dodecyl-benzene sulfonate - 0.808; benzalkonium chloride - 0.730; and chloroxylenol - 0.857) were found to be significant (*p*<0.05).

### Half maximal biofilm inhibitory concentration (BIC<sub>50</sub>) of *S. Typhimurium* biofilm

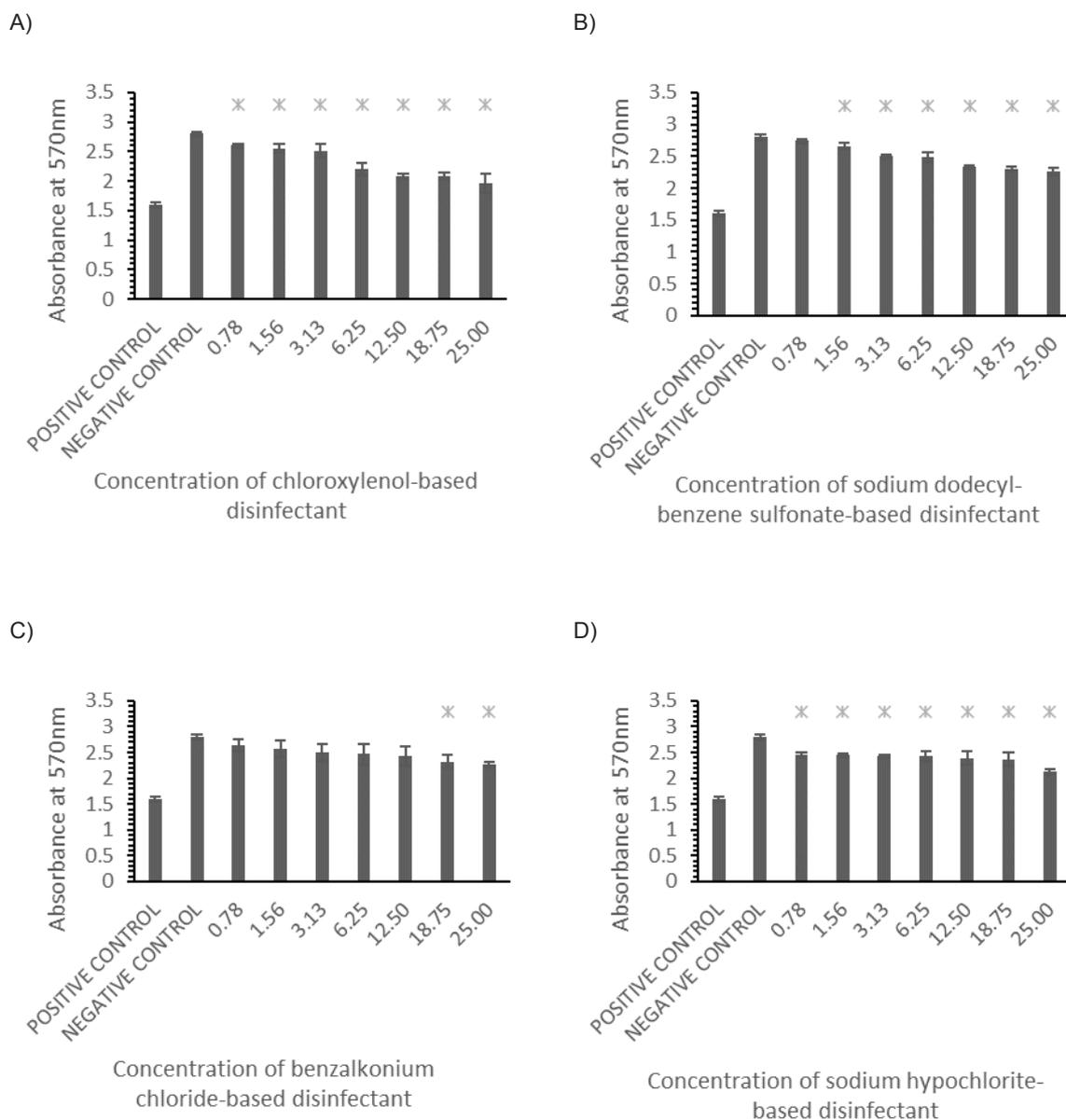
Table 2 displays the BIC<sub>50</sub> values of commercial disinfectants. The biofilm inhibition strength followed the order: chloroxylenol-based disinfectant > sodium hypochlorite-based disinfectant > sodium dodecyl-benzene sulfonate-based disinfectant > benzalkonium chloride-based disinfectant.

### FTIR spectra of *S. Typhimurium* biofilm

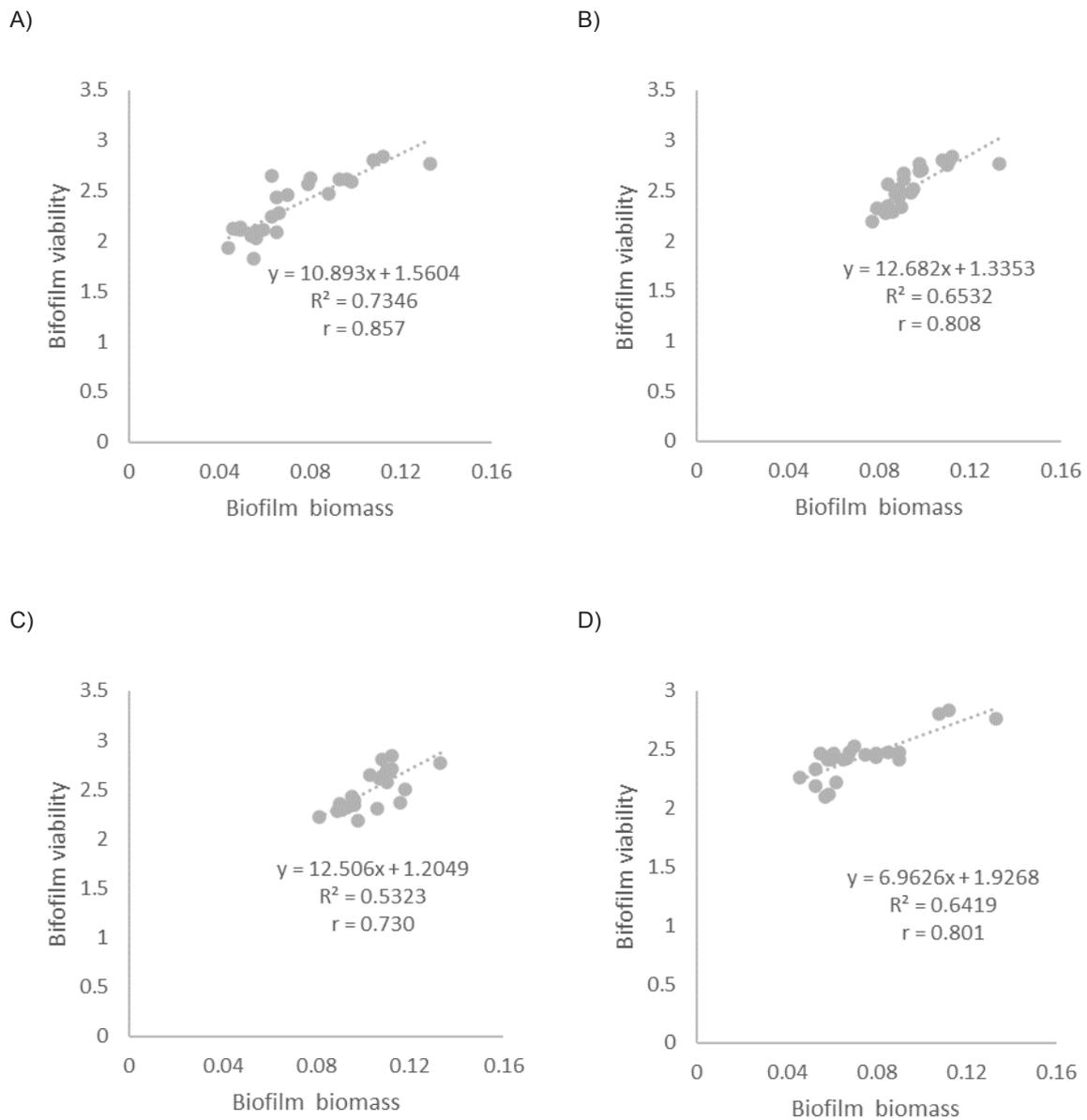
Table 3 shows IR assignments of functional groups corresponding to the major biomolecules in *S. Typhimurium* biofilm which are lipid, protein, nucleic acid, and polysaccharide. Meanwhile, Figure 4 shows the FTIR-ATR spectra of *S. Typhimurium* biofilm. Treatment with chloroxylenol-based disinfectants caused alteration in FTIR spectral peaks associated with lipid (1460 cm<sup>-1</sup>), protein (630 cm<sup>-1</sup>, 702 cm<sup>-1</sup>, 1550 cm<sup>-1</sup> & 1650 cm<sup>-1</sup>), and nucleic acid (1080 cm<sup>-1</sup> & 1229 cm<sup>-1</sup>). The biochemical modifications of *S. Typhimurium* biofilm were also consistent with the inhibitory effects as shown by the crystal violet and resazurin assays.



**Fig. 1.** Biomass of *S. Typhimurium* ATCC14028 biofilm treated with chloroxylenol-based disinfectant (A), sodium dodecyl-benzene sulfonate-based disinfectant (B), benzalkonium chloride-based disinfectant (C) and sodium dodecyl-benzene sulfonate-based disinfectant (D). Positive control: IP-protected antibiofilm cocktail while negative control: bacterial inoculum. Each bar represents the mean  $\pm$  standard deviation. Asterisks (\*) show significant differences ( $p < 0.05$ ) as compared to the negative control group.



**Fig. 2.** Viability of *S. Typhimurium* ATCC14028 biofilm treated with chloroxylenol-based disinfectant (A), sodium dodecyl-benzene sulfonate-based disinfectant (B), benzalkonium chloride-based disinfectant (C) and sodium hypochlorite-based disinfectant (D). Positive control: IP-protected antibiofilm cocktail while negative control: bacterial inoculum. Each bar represents the mean ± standard deviation. Asterisks (\*) show significant differences ( $p < 0.05$ ) as compared to the negative control group.



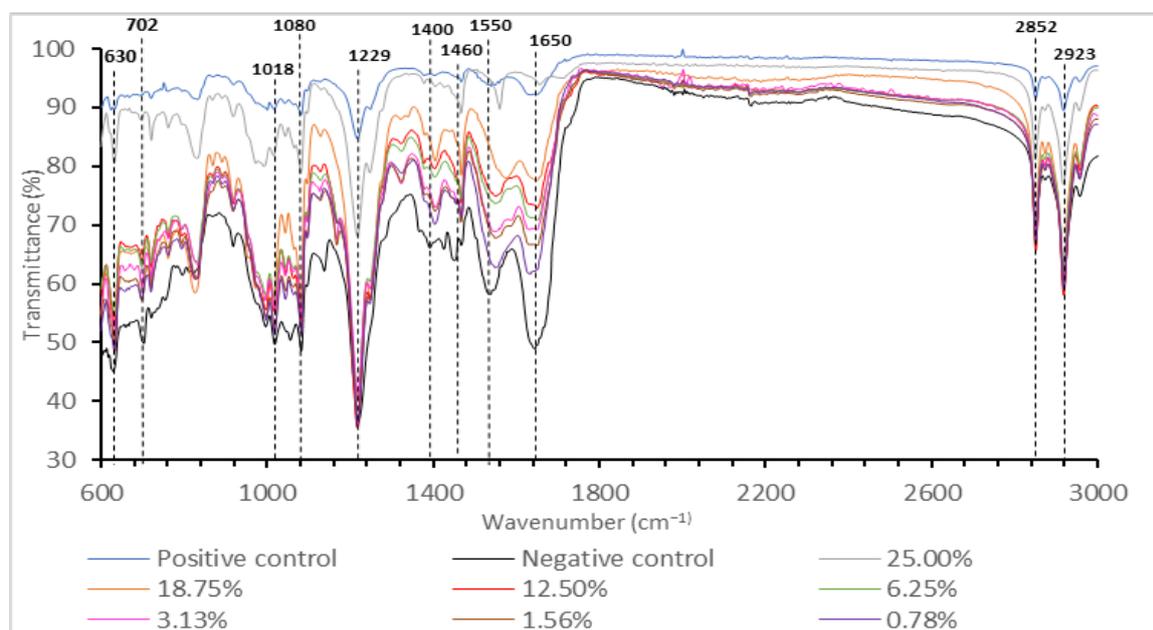
**Fig. 3.** Correlations between biomass and viability of *S. Typhimurium* ATCC14028 biofilm in the presence of commercial disinfectants. A: chloroxylenol-based disinfectant (coefficient correlation value: 0.857); B: sodium dodecyl-benzene sulfonate-based disinfectant (coefficient correlation value: 0.808); C: benzalkonium chloride-based disinfectant (coefficient correlation value: 0.730); D: sodium hypochlorite-based disinfectant (coefficient correlation value: 0.801).

**Table 2.** BIC<sub>50</sub> values obtained for the selected commercial disinfectants

Disinfectants	BIC <sub>50</sub> value (%)
Chloroxylenol-based disinfectant	5.06
Sodium dodecyl-benzene sulfonate-based disinfectant	9.58
Benzalkonium chloride-based disinfectant	14.68
Sodium hypochlorite-based disinfectant	5.33

**Table 3.** IR assignments of functional groups corresponding to the major biomolecules in *S. Typhimurium* biofilm

Wavenumber (cm <sup>-1</sup> )	IR assignment	Classification	References
2923	CH <sub>2</sub> asymmetric stretching	Lipid	Mester <i>et al.</i> , (2016)
2852	CH <sub>2</sub> symmetric stretching	Lipid	Mester <i>et al.</i> , (2016)
1650	C=O stretching and N-H bending (Amide I)	Protein	Biswas <i>et al.</i> , (2019)
1550	N-H bending and C-N stretching (Amide II)	Protein	DeQueiroz & Day (2007)
1460	C-H deformation of >CH <sub>2</sub>	Lipid & Protein	DeQueiroz & Day (2007)
1400	C=O symmetric stretching of COO <sup>-</sup> group	Lipid & Protein	DeQueiroz & Day (2007)
1229	P=O asymmetric stretching	Nucleic acid	Biswas <i>et al.</i> , (2019)
1080	P=O symmetric stretching and C-N-C stretching	Nucleic acid	Yahya <i>et al.</i> , (2018)
1018	C-O stretching	Polysaccharide	Yahya <i>et al.</i> , (2018)
702	N-H bending out of plane (Amide V)	Protein	Kalpna & Lee (2013)
630	O=C-N bending (Amide IV)	Protein	Gieroba <i>et al.</i> , (2020)

**Fig. 4.** FTIR spectra of *S. Typhimurium* ATCC14028 biofilm treated with chloroxylenol-based disinfectant. Spectral regions showing organic molecules in the biofilm, 600 – 3000 cm<sup>-1</sup>. Positive control: bacterial inoculum with IP-protected antibiofilm cocktail; Negative control: bacterial inoculum with fresh broth.

## DISCUSSION

Chloroxylenol, sodium dodecyl-benzene sulfonate, benzalkonium chloride, and sodium hypochlorite are disinfectants commonly used for disinfecting surfaces and cleaning medical devices. They generally function by disrupting the bacterial cell membrane and interfering with cellular metabolism. Herein, chloroxylenol-based disinfectant effectively hampered *S. Thyphimurium* biofilm. This result corroborates Bhathal (2018) showing the efficacy of chloroxylenol against microbial biofilms formed on denture base acrylic resin. The present study also revealed the inhibitory effect of benzalkonium chloride-based disinfectant against *S. Thyphimurium* biofilm. This finding is in agreement with Capita *et al.* (2019) demonstrating the efficiency of benzalkonium chloride in decreasing the percentage of surface covered by the biofilms of 10 strains of *Salmonella enterica*. The efficacy of sodium dodecyl-benzene sulfonate-based disinfectant against *S. Thyphimurium* biofilm was observed herein. Little is known about the effect of sodium dodecyl-benzene on other biofilms. However, other non-ionic surfactants such as sodium dodecyl sulfate is shown to eliminate *Salmonella* biofilm cells at the irreversible attachment phase (Wang *et al.*, 2016). Meanwhile, the present study demonstrated the efficacy of sodium hypochlorite-based disinfectant against *S. Thyphimurium* biofilm. This finding supports a previous work showing the inhibitory action of sodium hypochlorite against ten strains of *Salmonella enterica* (Capita *et al.*, 2019).

Pathogenic characteristics of microbial biofilm that cause diseases include viability, biomass, and extracellular matrix. In the present study, the correlation between biomass and viability of *S. Thyphimurium* biofilm in the presence of all commercial disinfectants was found to be significant. In 2018, Yahya *et al.* showed a significant correlation between the extracellular matrix and biomass of *S. Thyphimurium* biofilm treated with antimicrobial dimethyl sulfoxide. The synergistic inhibition of multiple pathogenic characteristics is important for successful biofilm control (Skogman *et al.*, 2012).

Half-maximal inhibitory concentration ( $IC_{50}$ ) is often used to measure the potential of a chemical substance to retard a specific biochemical function *in vitro*. The present study suggests chloroxylenol-based disinfectant as the best antibiofilm agent as it disrupts *S. Thyphimurium* biofilm significantly ( $p < 0.05$ ) at all test concentrations and shows the lowest  $BIC_{50}$  value. This suggestion contradicts other work showing that the sodium hypochlorite-based disinfectant represents the best disinfectant (Abdelaty, 2019). This disparity may be due to different surfaces for cellular attachment and different assay procedures.

Lipids are an important component of the extracellular matrix and are also found in the plasma membrane of a bacterial cell. It functions as protection for biofilm that is involved in biofilm growth and adhesion towards surfaces. In the present study, the peak at  $2923\text{ cm}^{-1}$ ,  $2852\text{ cm}^{-1}$ ,  $1460\text{ cm}^{-1}$ , and  $1400\text{ cm}^{-1}$  were assigned as lipid groups in the *S. Thyphimurium* biofilm spectra. The spectral peaks at  $2923\text{ cm}^{-1}$  and  $2852\text{ cm}^{-1}$  did not shift but showed a slight decrease in intensity as the concentrations of chloroxylenol-based disinfectant increased. This finding corroborates Mester *et al.* (2016) showing that FTIR peaks at  $2923\text{ cm}^{-1}$  and  $2852\text{ cm}^{-1}$  were not shifted following treatment of *S. Thyphimurium* with nalidixate ionic liquids.

Proteinaceous components play a role in surface colonization by biofilm and the formation of three-dimensional biofilm structures. Herein, the spectral peak at  $1650\text{ cm}^{-1}$ ,  $1550\text{ cm}^{-1}$ ,  $1460\text{ cm}^{-1}$ ,  $1400\text{ cm}^{-1}$ ,  $702\text{ cm}^{-1}$ , and  $630\text{ cm}^{-1}$  were assigned as protein groups in *S. Thyphimurium* biofilm spectra. A minor shifting happened at  $1650\text{ cm}^{-1}$  for Amide I to  $1640\text{ cm}^{-1}$  after treatment with sodium chloroxylenol-based disinfectant (0.78%). The intensity of this spectral peak also substantially decreased at all test concentrations. This is in line with Biswas *et al.* (2019) demonstrating that the spectral peak of *Acinetobacter baumannii* ATCC 19606 at  $1650\text{ cm}^{-1}$  was shifted to  $1634.78\text{ cm}^{-1}$  after the treatment with chlorhexidine-based disinfectant. The spectral peak at  $1550\text{ cm}^{-1}$  representing Amide II was found to shift to a lower frequency upon treatment with chloroxylenol-based disinfectant. DeQueiroz and Day (2007) showed that the FTIR spectral peak of  $1550\text{ cm}^{-1}$  disappeared in *P. aeruginosa* biofilm after exposure to a combination of sodium hypochlorite and hydrogen peroxide. The spectral peak at  $1460\text{ cm}^{-1}$  representing C-H deformation of  $\text{CH}_2$  in lipid and protein was found to disappear after treatment with chloroxylenol-based disinfectants (0.78% - 25%). Also, the spectral peak at  $1400\text{ cm}^{-1}$  representing symmetric stretching of C=O in amino acids and fatty acids was shifted to  $1410\text{ cm}^{-1}$  after treatment with chloroxylenol-based disinfectants (0.78%-25%) respectively. This is in line with DeQueiroz and Day (2007) reporting that FTIR spectral peak at  $1460\text{ cm}^{-1}$  disappeared while the spectral peak at  $1400\text{ cm}^{-1}$  shifted to  $1412\text{ cm}^{-1}$  in *P. aeruginosa* biofilm after exposure to a combination of sodium hypochlorite and hydrogen peroxide for 12 days.

Polysaccharides and nucleic acid are important components in extracellular polymeric substances that result in the initial attachment and biofilm structure of a bacterial cell. In the present study, the spectral peak at  $1229\text{ cm}^{-1}$ ,  $1080\text{ cm}^{-1}$ , and  $1018\text{ cm}^{-1}$  associated with nucleic acid and polysaccharide groups respectively were identified in *S. Thyphimurium* biofilm spectra. A spectral peak at  $1229\text{ cm}^{-1}$  did not show any spectral shift but showed a decrease in the intensity of the spectral peak at 25% of chloroxylenol-based disinfectant. This finding contradicts Biswas *et al.* (2019) reported that there was a shift in the FTIR signature peak from  $1229\text{ cm}^{-1}$  to  $1239\text{ cm}^{-1}$  of *Acinetobacter baumannii* ATCC 19606 after treatment with chlorhexidine-based disinfectant. This difference is probably due to the different types of bacteria and the type of disinfectant used. The spectral peaks at  $1080\text{ cm}^{-1}$  and  $1018\text{ cm}^{-1}$  did not show any spectral shift but showed a decrease in intensity as the concentrations of commercial disinfectants increased. This result is by Yahya *et al.* (2018) showing the decrease in FTIR peak intensity at  $1080\text{ cm}^{-1}$  and  $1018\text{ cm}^{-1}$  following treatment of *S. Thyphimurium* biofilm with dimethyl sulfoxide (DMSO).

Collectively, all FTIR spectral changes resulting from the treatment with chloroxylenol-based disinfectant observed herein indicate an altered structure of *S. Thyphimurium* biofilm (Yahya *et al.*, 2018; Ariafer *et al.*, 2019; Ojha & Ojha 2022; Kamaruzzaman *et al.*, 2022a). As FTIR spectroscopy provides real-time data and is sensitive to the structure, any change in the structure of the molecules is reflected in their spectroscopic fingerprint, peak position, and peak intensity. The present study may provide the first evidence of FTIR spectroscopy-based identification of the mode of action of chloroxylenol-based disinfectant against *S. Thyphimurium* biofilm.

## CONCLUSION

The present study demonstrated that all the selected commercial disinfectants effectually inhibited *S. Typhimurium* biofilm. Among all tested commercial disinfectants, chloroxylenol-based disinfectant was found to be the most effective against *S. Typhimurium* biofilm as it showed high inhibition of biomass and viability of *S. Typhimurium* biofilm at all test concentrations and the lowest BIC<sub>50</sub> value. It also caused changes in the composition of lipids, proteins, nucleic acids, and polysaccharides in *S. Typhimurium* biofilm. The findings of the present study suggest that the inhibition of chloroxylenol-based disinfectant against *S. Typhimurium* biofilm may involve structural changes of biofilm.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## REFERENCES

- Abdelaty, M., Nasr, S., Hamoud, M., Ismail, T., Laban, S., Gamal, A., Bashandy, E., Nasef, S. & Zahran, O. 2019. Efficiency of some sanitizers and disinfectants against biofilms and planktonic cells buildup on cages (galvanized wire) and plastic material (PVC) in poultry farms. *International Journal of Veterinary Science*, 8(3):120-126.
- Alves, D., Trevisan, C., Fiori, A., Negri, M., Alves, B., Filho, D.A. & Mikcha, G. 2015. Antibacterial and antibiofilm activity of carvacrol against *Salmonella enterica* serotype Typhimurium. *British Journal of Pharmaceutical Sciences*, 54(1): 1-8. <https://doi.org/10.1590/s2175-97902018000117229>
- Amamcharla, J.K., Panigrahi, S., Logue, C.M., Marchello, M. & Sherwood, J.S. 2010. Fourier transform infrared spectroscopy (FTIR) as a tool for discriminating *Salmonella typhimurium* contaminated beef. *Sensing and Instrumentation for Food Quality and Safety*, 4(1): 1-12. <https://doi.org/10.1007/s11694-009-9090-4>
- Ariafar, M.N., Iğci, N., Akçelik, M. & Akçelik, N. 2019. Investigation of the effect of different environmental conditions on biofilm structure of *Salmonella enterica* serotype virchow via FTIR spectroscopy. *Archives of Microbiology*, 201(9): 1233-1248. <https://doi.org/10.1007/s00203-019-01681-5>
- Bhathal, M., Kukreja, U. & Kukreja, N. 2018. Evaluation of efficacy of different denture disinfectants on biofilms formed on acrylic resin. *Dental Journal of Advance Studies*, 6(1): 020-027. <https://doi.org/10.1055/s-0038-1671696>
- Biswas, D., Tiwari, M. & Tiwari, V. 2019. Molecular mechanism of antimicrobial activity of chlorhexidine against carbapenem-resistant *Acinetobacter baumannii*. *PLoS ONE*, 14(10): 0224107. <https://doi.org/10.1371/journal.pone.0224107>
- Bressler, D., Balzer, M., Dannehl, A., Flemming, H.C. & Wingender, J. 2009. Persistence of *Pseudomonas aeruginosa* in drinking-water biofilms on elastomeric material. *Water Supply*, 9(1): 81-87. <https://doi.org/10.2166/ws.2009.026>
- Campos, J., Sousa, C., Mourão, J., Lopes, J., Antunes, P. & Peixe, L. 2018. Discrimination of non-typhoid *Salmonella serogroups* and serotypes by Fourier transform infrared spectroscopy: A comprehensive analysis. *International Journal of Food Microbiology*, 285: 34-41. <https://doi.org/10.1016/j.ijfoodmicro.2018.07.005>
- Capita, R., Fernández-Pérez, S., Buzón-Durán, L. & Alonso-Calleja, C. 2019. Effect of sodium hypochlorite and benzalkonium chloride on the structural parameters of the biofilms formed by ten *Salmonella enterica* serotypes. *Pathogens*, 8(3): 154. <https://doi.org/10.3390/pathogens8030154>
- DeQueiroz, G.A. & Day, D.F. 2007. Antimicrobial activity and effectiveness of a combination of sodium hypochlorite and hydrogen peroxide in killing and removing *Pseudomonas aeruginosa* biofilms from surfaces. *Journal of Applied Microbiology*, 103(4): 794-802. <https://doi.org/10.1111/j.1365-2672.2007.03299.x>
- Duygu, D., Udoh, A.U., Ozer, T. (Baykal), Akbulut, A., Ilkay (Acikgoz) Erkaya, Yildiz, K. & Guler, D. 2012. Fourier transform infrared (FTIR) spectroscopy for identification of *Chlorella vulgaris* Beijerinck 1890 and *Scenedesmus obliquus* (Turpin) Kützinger 1833. *African Journal of Biotechnology*, 11(16): 3817-3824. <https://doi.org/10.5897/AJB11.1863>
- Eguale, T., Marshall, J., Molla, B., Bhatiya, A., Gebreyes, W.A., Engidawork, E., Asrat, D. & Gunn, J. S. 2014. Association of multicellular behaviour and drug resistance in *Salmonella enterica* serovars isolated from animals and humans in Ethiopia. *Journal of Applied Microbiology*, 117(4): 961-971. <https://doi.org/10.1111/jam.12579>
- Eng, S.K., Pusparajah, P., Ab Mutalib, N.S., Ser, H.L., Chan, K.G. & Lee, L.H. 2015. *Salmonella*: A review on pathogenesis, epidemiology and antibiotic resistance. *Frontiers in Life Science*, 8(3): 284-293. <https://doi.org/10.1080/21553769.2015.1051243>
- Fouladynezhad, N., Afsah-Hejri, L., Rukayadi, Y., Nakaguchi, Y., Nishibuchi, M. & Son, R. 2013. Efficiency of four Malaysian commercial disinfectants on removing *Listeria monocytogenes* biofilm. *International Food Research Journal*, 20(3): 1485-1490.
- Garrett, T.R., Bhakoo, M. & Zhang, Z. 2008. Bacterial adhesion and biofilms on surfaces. *Progress in Natural Science*, 18(9):1049-1056. <https://doi.org/10.1016/j.pnsc.2008.04.001>
- Gieroba, B., Krysa, M., Wojtowicz, K., Wiater, A., Pleszczyńska, M., Tomczyk, M. & Sroka-Bartnicka, A. 2020. The FT-IR and Raman spectroscopies as tools for biofilm characterization created by cariogenic *Streptococci*. *International Journal of Molecular Sciences*, 21(11): 3811. <https://doi.org/10.3390/ijms21113811>
- Jean-Pierre, V., Boudet, A., Sorlin, P., Menetrey, Q., Chiron, R., Lavigne, J.P. & Marchandin, H. 2023. Biofilm formation by *Staphylococcus aureus* in the specific context of cystic fibrosis. *International Journal of Molecular Sciences*, 24(1): 597. <https://doi.org/10.3390/ijms24010597>

- Johari, N.A., Amran, S.S.D., Kamaruzzaman, A.N.A., Man C.A.I.C. & Yahya, M. F. Z.R. 2020. Anti-biofilm potential and mode of action of Malaysian plant species: A review. *Science Letters*, 14(2): 34-46. <https://doi.org/10.24191/sl.v14i2.9541>
- Kalpana, D. & Lee, Y.S. 2013. Synthesis and characterization of bactericidal silver nanoparticles using cultural filtrate of simulated microgravity grown *Klebsiella pneumoniae*. *Enzyme and Microbial Technology*, 52(3): 151-156. <https://doi.org/10.1016/j.enzmictec.2012.12.006>
- Kamaruzzaman, A.N.A., Mulok, T.E.T.Z. & Yahya, M.F.Z.R. 2022b. Inhibitory action of topical antifungal creams against *Candida albicans* biofilm. *Journal of Sustainability Science and Management*, 17(2): 27-34. <https://doi.org/10.46754/jssm.2022.02.003>
- Kamaruzzaman, A.N.A., Mulok, T.E.T.Z., Nor, N.H.M. & Yahya, M.F.Z.R. 2022a. FTIR spectral changes in *Candida albicans* biofilm following exposure to antifungals. *Malaysian Applied Biology*, 51(4): 57-66. <https://doi.org/10.55230/mabjournal.v51i4.11>
- Kart, D., Tavernier, S., Van Acker, H., Nelis, H.J. & Coenye, T. 2014. Activity of disinfectants against multispecies biofilms formed by *Staphylococcus aureus*, *Candida albicans* and *Pseudomonas aeruginosa*. *Biofouling*, 30(3): 377-383. <https://doi.org/10.1080/08927014.2013.878333>
- Lineback, C.B., Nkemngong, C.A., Wu, S.T., Li, X., Teska, P.J. & Oliver, H.F. 2018. Hydrogen peroxide and sodium hypochlorite disinfectants are more effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms than quaternary ammonium compounds. *Antimicrobial Resistance and Infection Control*, 5(1): 1-7. <https://doi.org/10.1186/s13756-018-0447-5>
- Mahat, M.M., Aris, A.H., Jais, U.S., Yahya, M.F., Ramli, R., Bonnia, N.N. & Mamat, M.T. 2012. A preliminary study on microbiologically influenced corrosion (MIC) of mild steel by *Pseudomonas aeruginosa* by using infinite focus microscope (IFM). *AIP Conference Proceedings*, 1455: 117-123. <https://doi.org/10.1063/1.4732479>
- Merino, L., Procura, F., Trejo, F.M., Bueno, D.J. & Golowczyc, M.A. 2019. Biofilm formation by *Salmonella* sp. in the poultry industry: Detection, control and eradication strategies. *Food Research International*, 119: 530-540. <https://doi.org/10.1016/j.foodres.2017.11.024>
- Mester, P., Jehle, A. K., Leeb, C., Kalb, R., Grunert, T. & Rossmanith, P. 2016. Ftir metabolomic fingerprint reveals different modes of action exerted by active pharmaceutical ingredient based ionic liquids (API-ILS) on *Salmonella typhimurium*. *RSC Advances*, 6(38): 32220-32227. <https://doi.org/10.1039/C5RA24970H>
- Meyer, B. & Cookson, B. 2010. Does microbial resistance or adaptation to biocides create a hazard in infection prevention and control? *Journal of Hospital Infection*, 76(3): 200-205. <https://doi.org/10.1016/j.jhin.2010.05.020>
- Mohamed, M.A., Jaafar, J., Ismail, A.F., Othman, M.H.D. & Rahman, M.A. 2017. Fourier transform infrared (FTIR) spectroscopy. In: *Membrane Characterization*. N. Hilal, A.F. Ismail, T. Matsuura & D. Oatley-Radcliffe (Eds.). Elsevier. pp. 3-29. <https://doi.org/10.1016/B978-0-444-63776-5.00001-2>
- Neu, T.R. & Lawrence, J.R. 2010. Extracellular polymeric substances in microbial biofilms. In: *Microbial Glycobiology*. O. Holst, P.J. Brennan, M. von Itzstein & A.P. Moran (Eds.). Academic Press. pp. 733-758. <https://doi.org/10.1016/B978-0-12-374546-0.00037-7>
- Nor, F.M., Aazmi, S., Anuar, T.S., Muslim, A., Aziz, M.N. Ibrahim, N., Raja Yahya, M.F.Z., Zainuri, S.N. & Mohd Yusof, F.Z. 2023. A laboratory perspective on an epidemiological pattern of infectious gastroenteritis: A five-year surveillance between 2016 to 2020 from established private healthcare centers within Klang Valley in Malaysia. *Journal of Pure and Applied Microbiology*. <https://doi.org/10.22207/JPAM.17.1.07>
- Ojha, S.K. & Ojha, A.K. 2022. Raman and FTIR spectroscopic techniques and their applications. *Upconverting Nanoparticles: From Fundamentals to Applications*. V. Rai (Ed.). Wiley. pp. 97-116. <https://doi.org/10.1002/9783527834884.ch4>
- Othman, N.A. & Yahya, M.F. 2019. In silico analysis of essential and non-homologous proteins in *Salmonella typhimurium* biofilm. *Journal of Physics: Conference Series*, 1349(1): 012133. <https://doi.org/10.1088/1742-6596/1349/1/012133>
- Preisner, O., Guiomar, R., Machado, J., Menezes, J.C. & Lopes, J.A. 2010. Application of fourier transform infrared spectroscopy and chemometrics for differentiation of *Salmonella enterica* serovar enteritidis phage types. *Applied and Environmental Microbiology*, 76(11): 3538-3544. <https://doi.org/10.1128/AEM.01589-09>
- Roth, G.A., Abate, D., Abate, K.H., Abay, S.M., Abbafati, C., Abbasi, N., Abbastabar, H., Abd-Allah, F., Abdela, J., Abdelalim, A., Abdollahpour, I., Abdulkader, R.S., Abebe, H.T., Abebe, M., Abebe, Z., Abeje, A.N., Abera, S.F., Abil, O.Z., Abraha, H.N. & Murray, C.J.L. 2018. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980-2017: A systematic analysis for the Global Burden of Disease Study 2017. *The Lancet*, 392(10159): 1736-1788. [https://doi.org/10.1016/S0140-6736\(18\)32203-7](https://doi.org/10.1016/S0140-6736(18)32203-7)
- Skogman, M.E., Vuorela, P.M. & Fallarero, A. 2012. Combining biofilm matrix measurements with biomass and viability assays in susceptibility assessments of antimicrobials against *Staphylococcus aureus* biofilms. *The Journal of Antibiotics*, 65(9): 453-459. <https://doi.org/10.1038/ja.2012.49>
- Tassinari, E., Duffy, G., Bawn, M., Burgess, C.M., McCabe, E.M., Lawlor, P.G., Gardiner, G. & Kingsley, R.A. 2019. Microevolution of antimicrobial resistance and biofilm formation of *Salmonella typhimurium* during persistence on pig farms. *Scientific Reports*, 9(1). <https://doi.org/10.1038/s41598-019-45216-w>
- Wang, H., Wang, H., Xing, T., Wu, N., Xu, X. & Zhou, G. 2016. Removal of *Salmonella* biofilm formed under meat processing environment by surfactant in combination with bio-enzyme. *LWT - Food Science and Technology*, 66: 298-304. <https://doi.org/10.1016/j.lwt.2015.10.049>
- Yaacob, M.F., Murata, A., Nor, N.H., Jesse, F.F. & Raja Yahya, M.F. 2021. Biochemical composition, morphology and antimicrobial susceptibility pattern of *Corynebacterium pseudotuberculosis* biofilm. *Journal of King Saud University - Science*, 33(1): 101225. <https://doi.org/10.1016/j.jksus.2020.10.022>

- Yahya, M.F., Alias, Z. & Karsani, S.A. 2017. Antibiofilm activity and mode of action of DMSO alone and its combination with afatinib against gram-negative pathogens. *Folia Microbiologica*, 63(1): 23-30. <https://doi.org/10.1007/s12223-017-0532-9>
- Zawawi, W.M.A.W.M., Ibrahim, M.S.A., Rahmad, N., Hamid, U.M.A. & Yahya, M.F.Z.R. 2020. Proteomic analysis of *Pseudomonas aeruginosa* biofilm treated with *Chromolaena odorata* extracts. *Malaysian Journal of Microbiology*, 16(2): 124- 133. <https://doi.org/10.21161/mjm.190512>

