OPEN ACCESS

IJABR

International Journal of Advanced Biotechnology and Research
ISSN 0976-2612, Online ISSN 2278–599X,
17, (3), 2026, p 43-55

Research Article

Efficacy of ImmunoSEB™ in Treating Bacterial Infection and Its Role as a Bio-Enhancer with Antibiotics

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Article Info
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Received: 03/07/2026
Accepted: 05/08/2026
Published: 14/08/2026

DOI: 10.5281/zenodo.21927510

Publisher's Note: IJABR Press stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract

Bacterial infections pose a serious therapeutic challenge, particularly in the context of rising antibiotic resistance. ImmunoSEB™, a systemic enzyme blend, has been proposed as a combination therapy to enhance host immune response and improve antibiotic efficacy. 80-Swiss albino mice were randomized into 4-groups (n = 20 each): Infected control, Ampicillin (250 mg/kg/day), ImmunoSEB™ (400 mg/kg/day, administered 14-days prior and post-infection), and ImmunoSEB™ + Ampicillin combination. Infection was induced with *E. coli* ATCC25922. Thigh swelling was monitored daily, and bacterial load was quantified for 5-days post-treatment. Control mice exhibited marked thigh enlargement (90.77%) and high bacterial load (96.17 ± 24.24 CFU/g). Antibiotic (ampicillin) monotherapy slightly reduced the infection, with thigh swelling increasing to 103.92% and bacterial load remaining high (100.42 ± 34.45 CFU/g, non-significant over control). ImmunoSEB™ alone reduced thigh swelling to 100% and significantly lowered bacterial load to 70.58 ± 27.28 CFU/g ($p<0.05$). The combination therapy produced most prominent results, thigh swelling decreased to 75.73%, and bacterial load was nearly eradicated (2.25 ± 1.17 CFU/g, $p < 0.001$), representing a significant reduction compared to control. ImmunoSEB™-Ampicillin combination displayed near complete bacterial clearance and substantial inflammation resolution, highlighting its potential as an effective adjunct to conventional antibiotics in resistant bacterial infections. While these results in a murine model are encouraging, further studies are warranted to validate the findings.

Key words: ImmunoSEB™, Ampicillin, *Escherichia coli* infection, Combination therapy, Antibiotic resistance.

1. Introduction

Infectious diseases represent one of the most significant challenges to global health, despite remarkable advances in medical science and public health infrastructure. Each year, millions of people die from bacterial, viral, and fungal pathogens, a burden that is particularly severe in the developing world, where access to health care and effective treatments is limited [1]. The persistence of infectious diseases reflects not only the adaptability of pathogens but also the limitations of current therapeutic strategies. Conventional antibiotics and antivirals are essential, but are increasingly compromised by

challenges such as treatment failure, side-effects and the gradual loss of drug responsiveness observed in some pathogens. These facts underscore the need for new approaches to support existing therapies and provide more sustainable solutions towards managing infection [2,3].

Bacterial pathogens can cause major diseases, and clinical impact of these infections ranges from mild discomfort to life threatening complications [4]. The presence of bacterial pathogens across diverse patient populations, combined with their ability to adapt to therapeutic activity, has made bacteria a primary focus of ongoing infection control research [5]. Treatment of bacterial infections has traditionally relied on antibiotics, which remain the foundation of therapy. However, clinical experience increasingly reveals that certain strains respond less effectively to standard drugs, leading to prolonged illness and recurrent infections. Reports of reduced antibiotic efficacy highlight the adaptability of bacterial pathogens and the challenge of relying solely on conventional treatments [6]. This emerging problem of reduced drug responsiveness highlights the critical importance of investigating alternative approaches that can reduce bacterial burden and promote recovery without contributing to the pattern of increasing resistance.

A promising field of research is the use of enzymes as therapeutic agents. Enzymes are natural biocatalysts involved in important physiological processes such as digestion and metabolism [7,8]. Their therapeutic potential is based on their ability to degrade complex molecules and interfere with pathogen survival [9]. Unlike antibiotics, which act on specific bacterial targets, enzymes often exert broader effects, degrading structural or nutritional components that bacteria rely upon [10-12]. This general mode of action makes them valuable in infection management, as pathogens are less likely to develop resistance against enzymatic degradation. Therefore, enzymes can be used as an adjunctive therapy, improving the action of conventional drugs and the overall results of treatment [13].

Enzymes with proteolytic activity play a crucial role in disrupting bacterial colonization. By degrading these proteins, enzymes reduce bacterial invasion and limit the ability of pathogens to establish infection [14]. In a similar way, enzymes targeting carbohydrate substrates degrade polysaccharides that serve either as nutrient reserves or as components of protective biofilms [15]. This degradation not only limits bacterial growth but also reduces the structural integrity of microbial communities. Together, these enzymatic activities contribute to lowering the microbial load, making infections more manageable and improving the effectiveness of conventional therapies.

Beyond the direct breakdown of bacterial components, enzymes can also interfere with processes essential for infection progression. Many pathogens, such as *E. coli*, depend on adhesion to host tissues as a critical first step in establishing infection. Enzymatic activity that disrupts these adhesive interactions can therefore restrict colonization and limit the spread of disease [16]. Moreover, enzymes may degrade extracellular debris and damaged tissue, which often serve as reservoirs that support bacterial growth. By degrading these supportive conditions, enzymes contribute to reducing the overall microbial burden [17]. Taken together, these mechanisms highlight the versatility of enzymatic interventions in combating infection, not by directly killing bacteria, but by making the environment unfavorable for pathogens to proliferate. The use of enzyme blends that combine different activities offers a wide range of mechanisms for infection control. Since infections involve multiple bacterial survival strategies, targeting several components simultaneously increases the likelihood of effective intervention. Formulations that incorporate multiple strategies like proteolytic, catalytic and carbohydrate degrading activities can act on different aspects of bacterial persistence, weakening their ability to cause disease [18-21]. This approach not only reduces bacterial

survival but also make conventional therapies more effective, offering a broader and more resilient solution for infection management.

The exploration of enzyme-based therapies in the context of bacterial infection is particularly relevant. While antibiotics remain essential, their limitations such as reducing efficacy, adverse side effects, and the growing threat of resistance, underscore the need for adjunctive therapy [22]. Enzymes offer a complementary approach by targeting critical bacterial survival mechanisms, including structural proteins, carbohydrate matrices, and adhesion processes [23]. By weakening these defense mechanisms, enzymatic interventions enhance the effectiveness of conventional treatments and contribute to more efficient infection clearance. Importantly, such approaches may also reduce dependence on antibiotics, thereby helping to preserve their effectiveness for future use.

ImmunoSEB™, a proprietary systemic enzyme blend, has emerged as a promising candidate for infection management. The present study aims to evaluate the potential of ImmunoSEB™ in experimental infection model, with a focus on its effects on pathogen clearance. By directly assessing bacterial survival and progression of infection, this study aims to provide a comprehensive understanding of how enzyme-based therapies can complement existing approaches to infection management. The significance of this investigation extends beyond the immediate scope of *E. coli*. If enzyme-based therapies demonstrate effectiveness in reducing infection severity, they could represent a trend shift in the treatment of bacterial diseases. Rather than relying exclusively on pathogen-targeted drugs, clinicians may be able to employ adjunctive strategies that control bacterial infection and enhance therapeutic efficacy. This approach aligns with the growing emphasis in infectious disease research on alternative interventions designed to overcome the limitations of antibiotics and address the broader challenge of reduced drug responsiveness. Furthermore, enzyme-based therapies may offer advantages in terms of safety and tolerability, particularly when compared with prolonged antibiotic use, which is frequently associated with adverse effects and disruption of the host microbiome.

2. Materials and Methods

2.1. Bacterial strain

The *E. coli* ATCC 25922 strain was cultured in nutrient broth under aseptic conditions until the logarithmic growth phase was reached. To ensure optimal virulence, the inoculum was standardized to contain approximately 2×10^9 bacteria per 0.2 mL of culture broth, which was used for intramuscular injection into the thigh muscle of experimental animals.

2.2. Investigational product and experimental design

Eighty Swiss albino mice were used in the present study, with both sexes included in equal proportion across all groups. Animals were maintained in a bacteria free environment using isolators throughout the experimental period. Standard laboratory conditions were ensured, with controlled temperature, humidity, and a 12 h light/dark cycle. All animals were acclimatized to the experimental conditions for one week prior to initiation of the study.

The investigational product, ImmunoSEB™, was provided by Specialty Enzymes and Probiotics, Chino, CA, USA. ImmunoSEB™ was administered according to the treatment regimens described below. The mice were divided into four groups, each containing twenty animals. Group I served as the infected control and received no treatment throughout the study duration (infection period plus a 5 day observation). Group II was infected and treated with antibiotic (Ampicillin) at 250 mg/kg/day for 5 consecutive days beginning immediately after day 4 measurement. Group III received ImmunoSEB™ at 400 mg/kg/day for 14 days prior to infection and continued treatment for 5 days post infection. Group IV was infected and treated with a combination of ImmunoSEB™ (400 mg/kg/day) and antibiotic (Ampicillin) (250 mg/kg/day) for 5 consecutive days, beginning 3-days after infection was established.

Table 1: Treatment Schedule of Experimental Animals

Group	Treatment
Group I	Control, Infected, No treatment
Group II	Infected, treated with antibiotic (Ampicillin) 250 mg/kg/day
Group III	400 mg/kg/day of ImmunoSEB™ for 14 days prior to infection → followed by continued treatment with ImmunoSEB™ post-infection
Group IV	Infected → treated with ImmunoSEB™ 400 mg/kg and Antibiotic (Ampicillin) 250 mg/kg/day

Thus, the total study duration comprised 14 days of pretreatment for group III, followed by infection and a subsequent 5 day treatment/observation period for all groups. The treatment schedule is summarized in Table 1.

Bacterial infection was induced by injecting 0.2 mL of a standardized *E. coli* suspension into the right thigh muscle of animals in Groups I, II, and IV, under strict aseptic precautions. Group III animals, which had been pre-treated with ImmunoSEB™, were also inoculated with the bacterial culture following the same procedure.

2.3. Thigh enlargement score

Thigh enlargement score evaluation procedure was adopted by Hunter et al. [24]. In brief, thigh swelling was measured daily using Vernier caliper. Measurements were recorded on days 1, 2, and 3 following bacterial inoculation but prior to initiation of treatment, and subsequently on days 1 to 5 of the treatment period. For clarity, day 4 post inoculation was designated as day 1 of the post treatment period, as treatment commenced immediately after thigh measurement on that day. Changes in thigh diameter were interpreted as indicators of infection severity and therapeutic response.

2.4. Antibacterial study

At the end of the five day treatment period, six animals from each group were sacrificed under humane conditions. Approximately 1 g of infected thigh tissue was excised, minced in sterile saline, and homogenized. Serial dilutions ranging from 10^{-1} to 10^{-6} were prepared under aseptic precautions. Twenty microliters of the 10-6 dilution was seeded onto nutrient agar plates, which were incubated at 37 ± 2°C for 24 h. Colony counts were performed to determine the bacterial load in each group, expressed as CFU per gram of tissue.

2.5. Statistical analysis

Data on thigh measurements and bacterial counts were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using Student's t test according to the methods described by Panse and Sukhatme [25]. Differences were considered statistically significant at $p \leq 0.05$.

3. Results

A total of eighty Swiss albino mice were included in the study, with equal representation of both sexes across all groups. All animals were acclimatized for one week prior to infection and maintained under isolator conditions throughout the experimental period. Following inoculation with *E. coli* ATCC25922, animals in the infected control group developed progressive swelling of the inoculated thigh, accompanied by reduced activity and dullness. Mortality was noted in control group, confirming successful establishment of infection. In contrast, animals treated with ampicillin alone showed partial improvement, while those pre-treated with ImmunoSEB™ exhibited attenuated clinical signs. Combination therapy demonstrated the most rapid recovery, with minimal mortality and restoration of normal behavior.

3.1. Thigh enlargement

Thigh enlargement measurements following *E. coli* inoculation were performed to measure edema formation, providing a clear indicator of infection severity and therapeutic response. A Vernier caliper was used to measure the thigh length of each animal in the control and

treatment groups each day, according to the method described by Hunter et al. [24].

Measurements were taken before inoculation and continued till the completion of the experiment. After the inoculum was injected, the length of the thigh was measured each day to observe changes in response to the infection and the treatment.

In the control group (Group 1), the mean thigh length on Day 1 (prior to inoculum injection) was 7.42 ± 0.38 mm. Following the injection of the bacterial culture, a progressive increase was observed, with values of 10.58 ± 0.38 mm, 10.67 ± 0.25 mm, and 10.83 ± 0.38 mm on Days 2, 3, and 4 (i.e. day 1 of post-treatment period) respectively (Table 2).

During the post-treatment phase, thigh length in the control group ranged between 9.67 ± 0.54 mm and 10.58 ± 0.47 mm. The significant increase compared to Day 1 clearly indicated inflammation induced by the bacterial inoculum. Table 3 presents the percentage enlargement in thigh length for control and treatment groups on Day 4 (prior to treatment initiation) and Day 5 (post-treatment), relative to Day 1 (pre-inoculum). In the control group, thigh enlargement reached 145.95% on Day 4 compared to baseline. By the termination day, enlargement was reduced to 132.47%, which may reflect the development of partial immunity in response to infection.

However, thigh length remained elevated compared to Day 1, consistent with persistent infection related changes. In Group II animals treated with Ampicillin at a dose of 250 mg/kg, the mean thigh length on Day 1 (prior to inoculum injection) was 6.92 ± 0.15 mm. Following bacterial infection, thigh length increased progressively to 11.83 ± 0.46 mm on Day 2, 12.00 ± 0.58 mm on Day 3, and 12.75 ± 0.62 mm on Day 4. Day 4 was designated as Day 1 of the post-treatment period, as treatment commenced immediately after measurement.

After treatment, a statistically non-significant reduction in thigh length was observed on Day 3 (11.50 ± 0.58 mm) and Day 4 (11.50 ± 0.67 mm). However, by Day 5, animals in the Ampicillin treated group exhibited an elevated thigh length, indicating a relapse of inflammation associated with bacterial infection. This pattern suggests that while a transient stabilization occurred immediately after treatment initiation, Ampicillin failed to sustain therapeutic efficacy, likely due to bacterial resistance, resulting in repeated inflammatory enlargement. Percentage enlargement analysis (Table 3) showed that thigh length increased by 184.25% on Day 4 compared to baseline (Day 1). By the termination day, enlargement further rose to 191.47%, underscoring the absence of Ampicillin treatment efficacy.

Animals in Group III received ImmunoSEB™ at a dose of 400 mg/kg, starting 14 days prior to infection, to evaluate its potential to induce immunity against bacterial challenge. On Day 1 (prior to inoculation), the mean thigh length was 7.83 ± 0.11 mm.

Table 2: Thigh Enlargement Score in Mice Infected with *E. coli*

Group	Pre-treatment (mm)			Post-Treatment (mm)				
	Day 1	**Day 2**	**Day 3**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
Group I	7.42 ± 0.38	10.58 ± 0.38	10.67 ± 0.25	10.83 ± 0.38	9.67 ± 0.54	10.58 ± 0.47	9.92 ± 0.63	9.83 ± 0.71
Group II	6.92 ± 0.15	11.83 ± 0.46	12.00 ± 0.58	12.75 ± 0.62	12.00 ± 0.62	11.5 ± 0.58	11.5 ± 0.67	13.25 ± 1.53*
Group III	7.83 ± 0.11	12.00 ± 0.32	11.25 ± 0.25	10.25 ± 0.25	10.75 ± 0.25	10.5 ± 0.01	10.5 ± 0.01	10.25 ± 0.25*
Group IV	7.83 ± 0.31	11.08 ± 0.38	10.83 ± 0.17	11.33 ± 0.42	10.17 ± 0.42	10.00 ± 0.18	9.00 ± 0.18	8.58 ± 0.30

*Indicates significant difference compared to pre-treatment values ($p<0.05$)

Table 3: Percent Enlargement in Thigh Size of Mice Infected with *E. coli*

Group	Pre-treatment (mm)				Post-treatment (mm)	Enlargement (%)	
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 1→Day 4	Day 1→Day 5
Group I	7.42 ± 0.38	10.58 ± 0.38	10.67 ± 0.25	10.83 ± 0.38	9.83 ± 0.71	145.96	90.77
Group II	6.92 ± 0.15	11.83 ± 0.46	12.00 ± 0.58	12.75 ± 0.62	13.25 ± 1.53*	184.25*	103.92
Group III	7.83 ± 0.11	12.00 ± 0.32	11.25 ± 0.25	10.25 ± 0.25	10.25 ± 0.25*	130.91*	100
Group IV	7.83 ± 0.31	11.08 ± 0.38	10.83 ± 0.17	11.33 ± 0.42	8.58 ± 0.30	144.7	75.73*

*Indicates significant difference compared to pre-treatment values (p<0.05)

Following *E. coli* infection, thigh length increased to 12.00 ± 0.32 mm on Day 2, 11.25 ± 0.25 mm on Day 3, and 10.25 ± 0.25 mm on Day 4 (Day 1 of the post-treatment period). Thigh length varied from 10.25 ± 0.25 mm to 10.75 ± 0.25 mm during the post-treatment phase, suggesting persistent inflammation in comparison to baseline. Both pre-treatment and continued administration of ImmunoSEB™ alone did not lead to a noticeable decrease in thigh enlargement once infection was established. Percentage enlargement analysis (Table 3) showed an increase of 130.91% on Day 4 compared to Day 1. This was statistically lower than the enlargement observed in the control group, suggesting that ImmunoSEB™ pre-treatment may have partially boosted immunity against infection. However, by the termination day, enlargement remained at 100.00%, showing no significant improvement compared to baseline and indicating that ImmunoSEB™ alone did not effectively suppress bacterial growth once infection was established. Overall, although pre-treatment with ImmunoSEB™ appeared to reduce the initial thigh enlargement compared with controls, no statistically significant reduction was observed at termination. This outcome suggests that ImmunoSEB™ provided only limited benefit in managing the progression of *E. coli* infection.

Animals in Group IV received a combination of ImmunoSEB™ (400 mg/kg) and Ampicillin (250 mg/kg), beginning on Day 4 after infection induction. On Day 1 (prior to inoculation), the mean thigh length was 7.83±0.31 mm. After *E. coli* infection, thigh length increased to 11.08 ± 0.38 mm on Day 2, 10.83 ± 0.17 mm on Day 3, and 11.33±0.42 mm on Day 4 (day 1 of post-treatment period).

During the post-treatment phase, thigh length ranged between 8.58 ± 0.30 mm and 11.33 ± 0.42 mm. The initial increase relative to Day 1 confirmed inflammation due to bacterial inoculation. However, unlike Groups II and III, Group IV animals showed a statistically significant reduction in thigh size by the termination day, with measurements decreasing from 11.33 ± 0.42 mm (pre-treatment) to 8.58 ± 0.30 mm. Percentage enlargement analysis (Table 3) revealed an increase of 144.70% on Day 4 compared to baseline. By the termination day, enlargement was reduced to 75.73%, representing a statistically significant decrease relative to controls. This reduction highlights the antibacterial effect of the drug combination.

3.2. Bacterial load analysis

After completing the 5 day treatment period, six animals from both the treatment and control groups were sacrificed, and blood samples were collected for bacterial load estimation. Countable colonies in each group were observed

only at the 10^{-6} dilution, which was therefore selected for statistical analysis. The bacterial counts for all experimental groups at this dilution are summarized in Table 4.

Table 4: Mean Bacterial Count on Day 5 of Treatment

Group	Mean Bacterial Count (CFU)
Group I	96.17 ± 24.24
Group II	100.42 ± 34.45
Group III	70.58 ± 27.28*
Group IV	2.25 ± 1.17**

*Indicates significant reduction compared to infected control ($p<0.05$). **Indicates highly significant reduction compared to infected control ($p<0.01$).

In the control group, the mean bacterial count was 96.17 ± 24.24. Animals in Group II, treated with Ampicillin at 250 mg/kg, showed a non-significant increase in bacterial count (100.42 ± 34.45) compared to controls. In contrast, Group III animals, pre-treated with ImmunoSEB™ for 14 days prior to bacterial challenge and continued for 5 days thereafter, exhibited a statistically significant reduction in bacterial count (70.58 ± 27.28; $p<0.005$). This reduction suggests that ImmunoSEB™ pre-treatment induced immunity, leading to enhanced bacterial clearance. The effect was comparable to that observed with thigh length of group III, which showed non-significant reduction indicating partially boosted immunity against infection. Most notably, Group IV animals treated with a combination of ImmunoSEB™ (400 mg/kg) and Ampicillin (250 mg/kg) demonstrated a dramatic and statistically significant reduction in bacterial count (2.25 ± 1.17; $p<0.001$) compared to control. This finding indicates a synergistic effect, where ImmunoSEB™ enhanced the efficacy of Ampicillin, resulting in near-complete suppression of bacterial growth.

Overall, while Ampicillin alone produced only a non-significant reduction, ImmunoSEB™ pre-treatment significantly lowered bacterial counts, and its combination with Ampicillin yielded the most pronounced antibacterial effect.

4. Discussion

This study explored the therapeutic potential of ImmunoSEB™, Antibiotic (ampicillin), and their combination in Swiss albino mice infected with *E. coli* ATCC 25922. The infection model was successfully established, as the untreated controls developed progressive thigh swelling, reduced activity, and mortality. In the control group, thigh enlargement increased progressively after inoculation, reaching a peak by Day 4 and remaining elevated throughout the post-treatment period. This trend reflects the inflammatory response to bacterial proliferation and is consistent with the findings of Flores-Villaseñor et al. [26] who reported that untreated bacterial inoculation in murine models produced significant systemic infection. The elevated bacterial counts in the control group further confirmed the severity of infection, aligning with Silvaderuiz et al. [27] who demonstrated that *E. coli* challenge in mice leads to sustained bacterial colonization unless therapeutic intervention is applied. Together, these outcomes establish a clear baseline against which treatment efficacy can be assessed.

Antibiotic (ampicillin) treatment alone produced only temporary improvement of thigh enlargement, with relapse observed during the later treatment phase. The absence of sustained improvement and increased bacterial load at the termination point indicate limited therapeutic efficacy. Similar challenges have been observed in previous studies, where conventional antibiotics failed to manage *E. coli* infections effectively. Kieckens et al. [28] contributed significantly to it by demonstrating that antibiotic exposure in *E. coli* may increase disease severity via increased release of Shiga toxins (Stx). Their study showed that bovine lactoferrin (bLF) lowered the levels of Shiga toxin in a way that depended on the concentration and broke down the receptor binding B-subunit, which made the toxin less effective. At non-toxic concentrations, bLF also protected Vero cells from verocytotoxicity, showing that it can neutralize virulence factors instead of simply preventing bacterial growth. Taken together, these findings highlight a

critical limitation of antibiotic monotherapy. The relapse seen in this study with Ampicillin monotherapy illustrates this major concern and supports the adjunctive therapy like lactoferrin or enzyme-based therapies that can simultaneously reduce bacterial load and counteract pathogenic toxins.

Mice pre-treated with ImmunoSEB™ exhibited a modest therapeutic effect, characterized by partial reduction in thigh enlargement and a significant decrease in bacterial counts. Although thigh measurements remained elevated relative to baseline, the marked reduction in bacterial load suggests that ImmunoSEB™ enhanced host immunity, thereby aiding bacterial clearance. These findings correspond with previous studies on naturally derived enzymes against pathogenic microbes. Previous study has shown and validated the dual role of ImmunoSEB™ in helping the restoration of immune resilience of rats under immunosuppression and an enhancer in normal physiology, and a fine-tuned enhancer of baseline immune response [29]. Abo-Kamar et al. [15] demonstrated that purified α-amylase from *Bacillus cereus* possesses antibacterial and antibiofilm activity against uropathogenic *E. coli*. The enzyme reduced biofilm thickness and viability, while downregulating fimH and papC virulence genes. Mechanistically, α-amylase degraded the extracellular polymeric substance (EPS) matrix by cleaving α-1,4-glycosidic bonds and disrupted quorum sensing signals, thereby impairing bacterial adhesion and virulence regulation.

The iron-binding capacity of lactoferrin enzymes is primarily responsible for their antimicrobial effect, as it deprives pathogens of essential nutrition and disrupts bacterial membranes by binding to lipopolysaccharide content. This mechanism of pathogen inhibition was discussed by Griffiths et al. [30], who demonstrated marked inhibition of *E. coli* and *Salmonella typhimurium*. Notably, lactoferrin suppressed enteric pathogens while leaving probiotic *Bifidobacteria* unharmed in co-culture conditions. In parallel, Flores-Villaseñor et al. [26] reported that lactoferrin and its synthetic chimera suppressed pathogenic *E. coli* O157:H7 by reducing bacterial presence in feces and preventing systemic spread to organs. This inhibition was accompanied by protection against tissue damage, as treated groups exhibited alleviated renal and hepatic lesions compared to untreated controls. The study further noted that the antibacterial action was linked to lactoferrin's ability to disrupt bacterial membranes, thereby impairing pathogen survival and limiting dissemination. These findings highlight how enzyme-based treatments directly compromise pathogen viability and reduce infection severity. In addition, Yekta et al. [31] demonstrated that both bovine and human lactoferrin inhibited the growth of *E. coli* O157:H7 and significantly reduced bacterial attachment to intestinal epithelial cells. The study emphasized that lactoferrin's activity was not purely bacteriostatic but also anti-adhesive, as it degraded type III secreted proteins EspA and EspB, which are essential for bacterial adherence and effacing lesions.

Moreover, Praveen et al. [32] demonstrated that bromelain, a proteolytic enzyme derived from pineapple, possesses antibacterial activity against multiple pathogens. The study highlighted that bromelain interferes with bacterial adhesion to host glycoprotein receptors and signalling pathways, including cyclic AMP, cyclic GMP, and calcium-dependent cascades, thereby impairing colonization and promoting bacterial clearance.

In contrast, animals receiving the combination of ImmunoSEB™ and the antibiotic (ampicillin) showed a more pronounced effect. In this group, thigh enlargement was significantly reduced, indicating reduced inflammation and tissue swelling. A parallel reduction in bacterial load was observed, as *E. coli* counts dropped sharply under combination treatment. Collectively, these findings indicate that the enzyme composition of ImmunoSEB™ possesses notable antibacterial activity and, when paired with a conventional antibiotic such as ampicillin, acts synergistically to enhance antibacterial efficacy against pathogenic

bacteria. These findings correspond with Hogan et al. [33] who demonstrated that enzymatic agents such as dispersin B, lysostaphin, α-amylase, V8 protease, and serrapeptase, when combined with conventional antibiotics like vancomycin or rifampicin, significantly enhanced antibacterial efficacy against pathogenic growth. While antibiotics alone had limited effect on biofilm viability, their activity was markedly improved in the presence of enzymatic agents. Notably, lysostaphin and serrapeptase achieved complete inactivation of biofilm-associated bacteria when used in combination with antibiotics, underscoring the synergistic potential of enzyme-antibiotic therapy. Hogan et al. [33] further highlighted that these enzymes act by disrupting biofilm matrix components such as polysaccharides, peptidoglycan, and fibrin scaffolds, thereby facilitating antibiotic penetration and bactericidal activity.

Similarly, Skrabkova et al. [34] demonstrated that lysozyme forms complexes with antibiotics such as amikacin and levofloxacin, providing further evidence of synergistic interactions between enzymes and conventional drugs. The study showed that levofloxacin binding was most favourable at the active center of the enzyme, as it protect the critical residues Asp52 and Glu35 responsible for enzymatic function. This allowed lysozyme to retain its antibacterial potential while enhancing antibiotic efficacy. These results highlight that enzyme-antibiotic complexes can act synergistically, with lysozyme facilitating drug binding and penetration, thereby improving pathogen inhibition and reducing bacterial survival. Poilvache et al. [35] further confirmed that pre-treatment with an enzymatic cocktail followed by antibiotic exposure resulted in a synergistic reduction of pathogens. The combination achieved significantly greater decreases in bacterial counts compared to antibiotics alone, highlighting the value of enzyme-antibiotic synergy in enhancing pathogen clearance.

Consistent with these observations, dos Anjos et al. [36] demonstrated that enzyme preparation of papain and bromelain exerted marked antibacterial effects against *Alicyclobacillus* species. The study highlighted that when applied in combination, these enzymes displayed synergistic activity, achieving greater inhibition than when used individually. This evidence strengthens the rationale that enzyme-based approaches, particularly when paired together, can enhance antibacterial outcomes by supporting more effective infection control. In continuation, Kono [17] demonstrated that catalase exhibited bactericidal activity against *E. coli* in a dose, time, and pH dependent manner. Mechanistically, the antibacterial activity was attributed to the decomposition of lipid hydroperoxides present in catalase preparations, generating alkoxyl and peroxyl radicals that mediated bacterial killing.

Furthermore, Mecikoglu et al. [37] reported that proteolytic enzyme treatment exhibited antibacterial potential, particularly when evaluated against resistant bacterial strains. Further, Abbas et al. [38] showed that bromelain derived from pineapple waste displayed antibacterial activity against clinically relevant pathogens. These observations emphasized that enzyme treatment contributed to pathogen suppression and improved therapeutic outcomes. Similarly, Hossain et al. [39] investigated amylase production from *Bacillus* strains and demonstrated its inhibitory effect against *E. coli* and fungal pathogens. The study confirmed that enzymatic preparations could reduce microbial growth, thereby supporting their role in pathogen suppression. Together, these studies reinforce the conclusion that enzyme-based therapeutics, when paired with conventional antibiotics, can enhance antibacterial activity through complementary mechanisms, ultimately enhancing pathogen clearance and mitigating infection severity.

Overall, the present findings validate earlier evidence that adjunctive therapies can enhance infection resolution, while extending their relevance to ImmunoSEB™ in bacterial infection models. The data suggest that ImmunoSEB™ alone provides partial benefit by priming host immunity, but its combination with Ampicillin yields superior outcomes through

synergistic mechanisms. This integrated evidence positions ImmunoSEB™ as a promising adjunct to conventional antibiotics, offering a potential strategy focused on reducing infection and mitigating its severity.

5. Conclusion

This study demonstrates that ImmunoSEB™, a systemic enzyme blend, offers significant therapeutic benefit in the management of *E. coli* infection. Administered as a monotherapy, ImmunoSEB™ reduced bacterial burden and moderated inflammation, underscoring its potential to strengthen host defences. Most notably, when combined with Antibiotic (Ampicillin), the enzyme blend achieved highest bacterial clearance and substantial resolution of infection related swelling, outcomes that were not attainable with antibiotic treatment alone. These findings highlight the potential of ImmunoSEB™ as effective adjunct to conventional antibiotics, particularly in the context of rising antimicrobial resistance. By complementing antibiotic action and restoring host resilience, ImmunoSEB™ may help reduce dependence on high-dose or prolonged antibiotic regimens, thereby mitigating risks of resistance development. While these results in a murine model are encouraging, further studies are warranted to validate efficacy, explore optimal dosing strategies, and assess long-term safety. Taken together, this work positions ImmunoSEB™ as a novel adjunctive therapy that bridges conventional pharmacology with host-directed approaches, offering a pathway toward more sustainable infection management.

Acknowledgement

The Authors would like to thank Specialty Enzymes and Probiotics, Chino, USA, for providing the supplement, and Advanced Enzymes Technologies Ltd., Thane for funding the study. The authors would also like to thank Dr. Abhijeet B. Muley and Dr. Tanushree Sarkar for continuous assistance in manuscript writing.

Conflict of interest

The authors declare that there are no conflicts of interest

Ethical statement

All animal procedures were conducted at Advanced Enzyme Technologies Ltd., Thane, Maharashtra, India, in accordance with applicable ethical guidelines for the care and use of laboratory animals.

Funding

This study was funded by Advanced Enzymes Technologies Ltd., Thane, Maharashtra, India.

Declaration of Non-Use of AI

The authors confirm that no artificial intelligence tools were used in this study.

References:

[1] Nii-Trebi, N.I. (2017), Emerging and neglected infectious diseases: insights, advances, and challenges. BioMed Research International. 2017(1), 5245021. doi: 10.1155/2017/5245021

[2] Muteeb, G., Rehman, M.T., Shahwan, M., Aatif, M. (2023), Origin of antibiotics and antibiotic resistance, and their impacts on drug development: A narrative review. Pharmaceuticals. 16(11), 1615. doi: 10.3390/ph16111615

[3] Salam, M.A., Al-Amin, M.Y., Salam, M.T., Pawar, J.S., Akhter, N., Rabaan, A.A., Alqumber, M.A. (2023). Antimicrobial resistance: a growing serious threat for global public health. In: Healthcare (Vol. 11, No. 13, p. 1946). doi: 10.3390/healthcare11131946

[4] Kim, B.Y., Kang, J., Kim, K.S. (2005), Invasion processes of pathogenic Escherichia coli. International Journal of Medical Microbiology. 295(6-7), 463-470. doi: 10.1016/j.ijmm.2005.07.004

[5] Naidoo, N., Zishiri, O.T. (2025), Presence, pathogenicity, antibiotic resistance, and virulence factors of Escherichia coli: a review. Bacteria. 4(1), 16. doi: 10.3390/bacteria4010016

[6] Mousa, N., Aiesh, B.M., Jomaa, R., Zouneh, Y., Namrouti, A., Abutaha, A., Zyoud, S.E. H. (2025), Antibiotic resistance profiles and risk factors of multidrug-resistant Escherichia coli in a large tertiary care hospital in a low-and middle-income country. Scientific Reports. 15(1), 26667. doi: 10.1038/s41598-025-12384-x

[7] Rathi, A., Potale, S., Vaze, R., Muley, A.B., Jadhav, S. (2024), In vitro simulated study of macronutrient digestion in complex food using digestive enzyme supplement. Heliyon. 10(9), e30250. Doi: 10.1016/j.heliyon.2024.e30250

[8] Muley, A.B., Thorat, A.S., Singhal, R.S., Babu, K.H. (2018), A tri-enzyme co-immobilized magnetic complex: process details, kinetics, thermodynamics, and applications. International Journal of Biological Macromolecules. 118, 1781-1195. Doi: 10.1016/j.ijbiomac.2018.07.022

[9] Supriya, C., Sivareddy, C., Basaveswarao, M., Harshita, E., & Yashwanthi, P. (2017). Anti-bacterial, anti-fungal and analgesic activity of papain conjugated quercetin. European Journal of Biomedical. 4(04), 280-285.

[10] Rybarczyk, J., Kieckens, E., Vanrompay, D., Cox, E. (2017), In vitro and in vivo studies on the antimicrobial effect of lactoferrin against Escherichia coli O157: H7. Veterinary Microbiology. 202, 23-28. doi: 10.1016/j.vetmic.2016.05.010

[11] Kim, J.S., Lim, M.C., Kim, S.M., Lee, J.Y. (2023), Extracellular matrix-degrading enzymes as a biofilm control strategy for food-related microorganisms. Food Science and Biotechnology. 32(12), 1745. doi: 10.1007/s10068-023-01373-3

[12] Saini, S., Yadav, R., Das, R., Singh, C. (2026), Enzyme therapy as a promising strategy to overcome multi-drug resistance in pathogens: current advances, key challenges, and future directions. RSC Advances. 16(6), 5578-5605. doi: 10.1039/d5ra06932g

[13] Woo, S., Fogliano, V., Scala, F., Lorito, M. (2002), Synergism between fungal enzymes and bacterial antibiotics may enhance biocontrol. Antonie Van Leeuwenhoek. 81(1), 353-356. doi: 10.1023/a:1020540818163

[14] Salinas Ibáñez, Á.G., Vallés, D., Adaro, M., Barberis, S., Vega, A.E. (2021). Antimicrobial effect of a proteolytic enzyme from the fruits of Solanum granuloso-leprosum (Dunal) against Helicobacter pylori. Frontiers in Nutrition. 8, 699955. doi: 10.3389/fnut.2021.699955

[15] Abo-Kamar, A.M., Mustafa, A.E.R.A., Al-Madboly, L.A. (2024), Purified α-Amylase from Bacillus cereus exhibits antibiofilm and antiquorum sensing activities against uropathogenic Escherichia coli, downregulating FimH, and PapC virulence genes: implications for urinary tract infections. BMC Microbiology. 24(1), 502. doi: 10.1186/s12866-024-03542-8

[16] Mitrofanova, O., Mardanova, A., Evtugyn, V., Bogomolnaya, L., Sharipova, M. (2017), Effects of Bacillus serine proteases on the bacterial biofilms. BioMed Research International. 8525912. doi: 10.1155/2017/8525912

[17] Shin, K., Yamauchi, K, Teraguchi, S., Hayasawa, H., Tomita, M., Otsuka, Y., Yamazaki, S. (1998), Antibacterial activity of bovine lactoferrin and its peptides against enterohaemorrhagic Escherichia coli O157: H7. Letters in Applied Microbiology. 26(6), 407-411. doi: 10.1046/j.1472-765x.1998.00358.x

[18] Ariaeenejad, S., Zeinalabedini, M., Sadeghi, A., Gharaghani, S., Mardi, M. (2024), Enhancing nutritional and potential antimicrobial properties of poultry feed through encapsulation of metagenome-derived multi-enzymes. BMC Biotechnology. 24(1), 76. doi: 10.1186/s12896-024-00904-y

[19] Kono, Y. (1995), Apparent antibacterial activity of catalase: Role of lipid hydroperoxide contamination. The Journal of Biochemistry. 117(1), 42-46. doi: 10.1093/oxfordjournals.jbchem.a124718

[20] Roheen, T., Bibi, M., Rehman, M.F.U., Assad, N., Gondal, H.Y., Nadeem, M.,

Shahzad. (2025), Enhanced antibacterial and anti-inflammatory efficiency of serratiopeptidase immobilized on CMC-silver nanoparticles. Journal of Basic Microbiology. 65(11), e70060. doi: 10.1002/jobm.70060

[21] Epaud, R., Delestrain, C., Weaver, T.E., Akinbi, H.T. (2019), Bacterial killing is enhanced by exogenous administration of lysozyme in the lungs. Respiratory Medicine and Research. 76, 22-27. doi: 10.1016/j.resmer.2019.07.005

[22] Poirel, L., Madec, J.Y., Lupo, A., Schink, A.K., Kieffer, N., Nordmann, P., Schwarz, S. (2018), Antimicrobial resistance in Escherichia coli. Microbiology Spectrum. 6(4), 10-1128. doi: 10.1128/microbiolspec.ARBA-0026-2017

[23] Yamauchi, K., Tomita, M., Giehl, T.J., Ellison 3rd, R.T. (1993), Antibacterial activity of lactoferrin and a pepsin-derived lactoferrin peptide fragment. Infection and Immunity. 61(2), 719-728. doi: 10.1128/iai.61.2.719-728.1993

[24] Hunter, P.A., Rolinson, G.N., Witting, D.A. (1973), Comparative activity of amoxycillin and ampicillin in an experimental bacterial infection in mice. Antimicrobial Agents and Chemotherapy. 4(3), 285-293. doi: 10.1128/AAC.4.3.285

[25] Panse, V.G., Sukhatme, P.V. (1967), Statistical methods of agricultural workers. 2nd Endorsement. ICAR Publication, New Delhi, India, 381.

[26] Flores-Villaseñor, H., Canizalez-Román, A., Velazquez-Roman, J., Nazmi, K., Bolscher, J.G., Leon-Sicairos, N. (2012), Protective effects of lactoferrin chimera and bovine lactoferrin in a mouse model of enterohaemorrhagic Escherichia coli O157: H7 infection. Biochemistry and Cell Biology. 90(3), 405-411. doi: 10.1139/o11-089

[27] Silvaderuiz, C., Lopezdebocanera, M.E., Naderdemacias, M.E., Pescederuizholgado, A.A. (1996), Effect of lactobacilli and antibiotics on E. coli urinary infections in mice. Biological and Pharmaceutical Bulletin. 19(1), 88-93. doi: 10.1248/bpb.19.88

[28] Kieckens, E., Rybarczyk, J., Barth, S.A., Menge, C., Cox, E., Vanrompay, D. (2017), Effect of lactoferrin on release and bioactivity of Shiga toxins from different Escherichia coli O157: H7 strains. Veterinary Microbiology. 202, 29-37. doi: 10.1016/j.vetmic.2016.03.013

[29] Griffiths, E.A., Duffy, L.C., Schanbacher, F.L., Dryja, D., Leavens, A., Neiswander, R.L., Ogra, P. (2003), In vitro growth responses of Bifidobacteria and enteropathogens to bovine and human lactoferrin. Digestive Diseases and Sciences. 48(7), 1324-1332. doi: 10.1023/a:1024111310345

[30] Rathi, A., Rathi, V.L. (2026), ImmunoSEB™ enhances immune resilience: Hematological, humoral, and microarray evidence in normal and immunosuppressed Wistar rats. International Journal of Advanced Biochemistry Research. 10(6): 296-307. doi: 10.33545/26174693.2026.v10.i6d.8612

[31] Yekta, M.A., Verdonck, F., Broeck, W.V.D., Goddeeris, B.M., Cox, E., Vanrompay, D. (2010), Lactoferrin inhibits E. coli O157: H7 growth and attachment to intestinal epithelial cells. Vet Med – Czech. 55(8), 359-368. doi: 10.17221/2954-VETMED

[32] Praveen, N.C., Rajesh, A., Madan, M., Chaurasia, V.R., Hiremath, N.V., Sharma, A.M. (2014), In vitro evaluation of antibacterial efficacy of pineapple extract (bromelain) on periodontal pathogens. Journal of International Oral Health. 6(5), 96.

[33] Hogan, S., Zapotoczna, M., Stevens, N.T., Humphreys, H., O'Gara, J.P., O'Neill, E. (2017), Potential use of targeted enzymatic agents in the treatment of Staphylococcus aureus biofilm-related infections. Journal of Hospital Infection. 96(2), 177-182. doi: 10.1016/j.jhin.2017.02.008

[34] Skrabkova, H.S., Chernysheva, M.G., Baygildiev, T.M., Shnitko, A.V.,

Kasperovich, A.V., Egorova, T.B., Rodin, I.A. (2024), Lysozyme binding with amikacin and levofloxacin studied by tritium probe, fluorescence spectroscopy and molecular docking. Archives of Biochemistry and Biophysics. 751, 109848. doi: 10.1016/j.abb.2023.109848

[35] Poilvache, H., Ruiz-Sorribas, A., Cornu, O., Van Bambeke, F. (2021), In vitro study of the synergistic effect of an enzyme cocktail and antibiotics against biofilms in a prosthetic joint infection model. Antimicrobial Agents and Chemotherapy. 65(4), 10-1128. doi: 10.1128/AAC.01699-20

[36] dos Anjos, M.M., da Silva, A.A., de Pascoli, I.C., Mikcha, J.M.G., Machinski Jr, M., Peralta, R.M., de Abreu Filho, B.A. (2016), Antibacterial activity of papain and bromelain on Alicyclobacillus spp. International Journal of Food Microbiology. 216, 121-126. doi: 10.1016/j.ijfoodmicro.2015.10.007

[37] Mecikoglu, M., Saygi, B., Yildirim, Y., Karadag-Saygi, E., Ramadan, S.S., Esemenli, T. (2006), The effect of proteolytic enzyme serratiopeptidase in the treatment of experimental implant-related infection. The Journal of Bone & Joint Surgery, 88(6), 1208-1214. doi: 10.2106/JBJS.E.00007

[38] Abbas, S., Shanbhag, T., Kothare, A. (2021), Applications of bromelain from pineapple waste towards acne. Saudi Journal of Biological Sciences. 28(1), 1001-1009. doi: 10.1016/j.sjbs.2020.11.032

[39] Hossain, K.M., Khan, U., Rahman, S.M., Khan, M.S. (2024), Potential antimicrobial and fruit juice clarification activity of amylase enzyme from Bacillus strains. Biotechnology Reports. 44, e00861. doi: 10.1016/j.btre.2024.e00861