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Antibacterial Activity and *in-vivo* Protection Potentials of *Aspergillus* Species SK2 Antibiotic Substance against the Establishment of Infections By β- Lactamase Producing Clinical Bacteria

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ABSTRACT

Antibacterial activity of an *Aspergillus* species SK2 (AS – SK2) antibiotic substance against β-lactamase producing bacteria assayed by agar-well diffusion technique, indicated broad-spectrum of activity with significant ($P < 0.05$) high potencies compared with the controls against *Staphylococcus aureus*(SA): *Staphylococcus epidermides, Staphylococcus saprophyticus* (SS); β-lactamase-positive (methicillin-resistant) *Staphylococcus aureas* (MRSA); *Bacillus subtilis (*BS*); Streptococcus pyogenes* (SP); *Escherichia coli* (EC)*' Salmonella typhi* (ST)*; Pseudomonas aeruginosa*(PA); *Shigella dysentariae* (SD); *Vibrio cholerae* (VC) *Klebsiella pnuemoniae* (KP)*; Proteus mirabilis* (PM).The mode of activity of the agent assayed by macrobroth dilution technique, indicated bacteriostatic activity with high stability or potency to β- lactamase activity and relatively low MIC (0.06 mg/ml P.E) for SS, MRSA, SP, ST, SD and VC; but higher for both EC (0.24 mg/ml P.E) and PA (0.48 mg/ml P.E) respectively compared with the control. The antibiotic substance was relatively non-toxic with an LD_{50} of 3.8 mg/ml P.E/Kg and a large therapeutic index of 7.92. The AS-SK2 agent offered high rate of protection to the experimental animals challenged intraperitoneally with both the pathogens and antibiotics substances against the successful establishment of the infections by the β- lactamase producing Gram-positive and Gram-negative bacteria. Of particular interest is the activity against the MRSA which recorded 100% protection, while EC only recorded 33.3% protection and PA recorded no protection but 100% mortality.

Keyword: Antibacterial Activity, *Aspergillus* species SK2, Antibiotic substances, β- lactamase producing staphylococci, and Mouse protection test (MPT).

INTRODUCTION

Of the several hundreds of antibiotics purified and characterized, only very few have been significantly useful as chemotherapeutic agents. This is primarily due to the twin-problems of toxicity to host tissues and the ever-increasing bacterial resistance to antibiotics. Together, these problems pose a major set-back to the usefulness of antibiotics as chemotherapeutic agents. Obviously, antibiotics resistance in clinical bacteria has been widely reported to be the second most disturbing problem in health care delivery after toxicity, accounting for up to 90% of infections (Davis, 1979; Neu, 1983, Neu, 1984 Davies, 1984). Clinical and nosocomial bacteria continue to evolve antibiotic resistance mechanisms, which enable them to grow and multiply even in the presence of high concentrations of antibiotics, as well as other antimicrobics, resulting in elevated minimum inhibitory concentrations (MIC) of antibiotics. The resulting high MICs render antibiotics ineffective and useless as chemotherapeutic agents due to elevated antibiotics target active index, (TAI) (Nikaido and Normark, 1987; Nikaido, 1989). Bacterial resistance to antibiotics could be either intrinsic (inherent) or non-intrinsic (acquired). The acquired resistance is a result of spontaneous chromosomoal mutations, or acquisition of extrachromosomal elements like plasmids, transposons or integrons by the processes of genetic transfers. The resultant genetic alterations always give rise to the several biochemical mechanisms of bacterial resistance to antibiotics, chiefly amongst which is the elaboration of many antibiotics inactivating or destroying enzymes, notably the β-lactamases. β-lactamases are bacterial enzymes which have important role in bacterial resistance to the β-lactams, and other antibiotics by opening the β-lactam ring of these generic antibiotics, thereby inactivating the β- lactams as well as other antibiotics through extended spectrum of β-lactamase activity. Thus, the production of β-lactamases, is a major mechanism of resistance of Gram-positive and Gram-negative bacteria to antibiotics (Skye and Matthew, 1976; Brown and Reynolds, 1980; Sanders and Sanders, 1985; Pulverer, *et al.*, 1986).

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The elaboration of β-lactamases-mediated resistance in bacteria has evolved increased prevalence in certain organisms, notably *Staphylococcus aureus*, and spread to new hosts of both Gram-positive and Gram-negative bacteria such as *Haemophilus influenzae, N. gonorrhea, E. faecalis, K. pnuemoniae* and other Enterobacteriaceae (Pulverer *et al.*, 1986; Brown and Reynolds, 1980; Roy *et al.*, 1985; Spencer, *et al.*, 1987; Sirot *et al.*, 1988; Phillipon *et al.*, 1989; Main *et al.*, 1999). Bacterial β- lactamases, which are closely related to the penicillin-binding proteins, (PBP) (Maloun and Bryan, 1986; Massiva and Mobashery, 1998;), frequently caused alterations in the target-sites of β- lactam antibiotics resulting in a decreased in amount or loss of affinity for β- lactams of a crucial PBP, hence the resistance (Maloun and Bryan, 1986). The evolution ofβ- lactamases presented a distinct survival advantage to the bacterial producers than the non-producers. Hence, the presence of the antibiotic creates the selective pressure for their survival and dissemination of their genetic materials (Massiva and Mobashery, 1998). This process has been accelerated considerably by the medical use of antibiotics, since β- lactams were discovered and used heavily for the treatment of wound infections since World War II (Davies, 1994; WHO, 1996). The enzymes which are usually readily transmissible have been reported to carry resistance to the aminoglycosides, tetracyclines, macrolides, quinolones as well as many other antibiotics classes (Sanders and Sanders-Jr. 2005; Sanders and Watakunakorn, 1986; Spencer *et al.*, 1987; Sirot *et al.*, 1988; Philipon *et al.*, 1989; Pena *et al.* 1998, Patterson *et al.*, 2004). Thus, in β-lactam research, a major problem is to evade the action of these inactivating enzymes; and several methods have been reported (Sanders and Sanders, 1985; Lobia *et al.*,1986; Pulverer *et al.*, 1986). One method is the design of β-lactamase-resistant,β-lactams. However, an appropriate balance between β-lactamase resistance and good antibacterial activity is often difficult to achieve. Another method is to dissociates these two aspects and combine a well-known β- lactam antibiotic with an efficient β-lactamase inhibitor. Thus, with the seemingly exponential emergence of clinical bacteria becoming resistant to most of the clinically available antibiotics already marketed, the need for discovering new and novel antibiotics, particularly from natural sources, becomes highly imperative. This present paper, had examined the degree of antibacterial activity as well as the protection potential of *Aspergillus* SK2 antibiotic substance against the establishment of infections by β- lactamase producing staphylococci as well as other clinical bacterial species.

MATERIALS AND METHODS

Microorganisms, Culture Conditions and Inoculum standardization

The antibiotic producing *Aspergillus* species SK2, previously isolated from Nsukka soil, Nigeria was grown an Sabouraud dextrose agar, (SDA)(Oxoid, England) plates containing 0.5 mg/ml chloramphenicol at 28 ^{o}C for 48 h (Ekong *et. al.*, 2004). The cell was maintained at 4 ^{o}C in SDA slants of the stated composition. The test organisms used in the study were clinical bacterial cultures comprising of β-lactamase producing (methicillin-resistant) *Staphylococcus aureus* (MRSA); *Staphylococcus aureus*(SA); *Staphylococcus epidermides* (SE); *Staphylococcus saprophyticus*(SS); *Bacillus substilis* (BS); *Escherichia coli* (EC): *Pseudomonas aeruginosa*(PA); *Salmonella typhi* (ST); *Klebsiella pneumonia* (KP). They were obtained from the University of Nigeria Teaching Hospital (UNTH), Enugu, Nigeria. The cultures were grown on plates of nutrient-agar, (NA) (Oxoid, England), incubated at 37 ^{o}C for 24 h and maintained by sub-culturing in the stated medium slants, stored at 4 ^{o}C. Inocula of the test cultures were standardized to that of 0.5 MacFarlane and Nephelometer, with approximate cell-density of $1.5x10^{8}$ cfu/ml following the methods of Tilton and Howard, (1987); Baron and Finegold, (1990); with modifications, Ekong *et al.*,(2004). The antibiotic producing culture and those of Gram-positive bacteria were ten-fold serially diluted to factor 3, while those of Gram-negative bacteria were diluted to factor 5 (Ekong *et al.*, 2004).

Antibiotic Production

Cultures of *Aspergillus* species, SK2 (AS – SK2) were grown aerobically under submerged fermentation conditions in 250 ml capacity Erlenmeyer flasks containing 150 ml Woodward medium, (WM) (Glycerol 1%; yeast extract 2%), as previously reported (Ekong *et al.*, 2004). The WM was inoculated with 1.0 ml standard suspension of the antibiotic producing strain and fermentation was carried out using orbital shaking incubator (Gallenkamp, England), operating at 100 rpm and 28 ^{o}C for 14 days. Cells were harvested by centrifugation at 8,000xg for 15 mins, in an RC5 – B centrifuge (Ivan Sorvall, Inc, Norwalk, USA). The supernatants fractions obtained were aseptically filtered with a sterile-filtration apparatus containing an acrodisc-filter (0.45 μm pore size).The filtrates obtained were referred to as the crude antibiotic solution and were aseptically stored in Bijou bottles at 4^{o}C.

Antibacterial Activity Tests

The susceptibility of the clinical bacterial cultures to the AS-SK2 crude antibiotic solution was investigated by the standard agar- well diffusion technique (Collins and Lyne, 1979), with modification Ekong *et al.,* (2004). To wells bored on plates of diagnostic sensitivity test agar, DSTA (Oxoid, England), previously seeded by spread-plating 0.1 ml standardized incocula of the test cultures were aseptically filled with 0.2 ml of the crude antibiotic solution. The assay plates were held at 4 ^{o}C for 1 h, to slow down bacterial growth and allow increased diffusion of the antibiotics solution into the assay medium (Ekong *et al.,* 2004). Antibacterial susceptibility evident by the presence of inhibitory zones was observed after incubation for 24 h at 37 ^{o}C. The minimum inhibitory concentration (MIC) of the antibiotic substance against the clinical bacteria tested was assayed using the macro broth-dilution technique (Tilton and Howard, 1987; Baron and Finegold, 1990) with modifications (NCCLS, 1997; Ekong *et al.,* 2004). Tubes containing two-fold serially diluted antibiotic solutions with diagnostic sensitivity test broth (DSTB) were inoculated with 0.1 ml standardized bacterial culture broths, and incubated at 37^{o}C for 24 h for growth. Uninocolated DSTB served as controls. The MIC of the AS -SK2 antibiotic solution was taken as the least concentration of the antibiotic substance that inhibited the growth of the test organisms. The minimum bactericidal concentration (MBC) of the AS-SK2 antibiotic solution, were determined from 24 h further incubation of the non-turbid MIC tubes at standard conditions for bacteria. The tubes were observed for the presence or absence of turbidity. Thereafter, the MBC was taken as the least concentration of the antibiotic substance without growth, from which the mode or activity was determined (Ekong *et al.,* 2004).

Relative Quantitation of Antibiotic Substance

Relative quantitation of amount (concentration) of antibiotic in the antibiotic solution was based on comparable levels of inhibition of *Stpahylococcus aureus* by penicillin G, assayed by agar-well diffusion technique (Barja *et al.,* 1989), with modifications (Ekong *et al.,* 2004). The corresponding inhibition zones obtained in the penicillin G sensitivity were used to construct a penicillin-G standard curve, from which the concentration of antibiotic solution was determined and thereafter expressed as mg/ml of penicillin G equivalent (mg/ml P.E). One unit of antibacterial activity was arbitrarily taken as the quantity of the antibiotic substance given rise to the same level of inhibition as 0.19 mg/ml penicillin G (Ekong *et al*., 2004).

Acute Toxicity (LD_{50}) of AS-SK2 Antibiotics Substance

The acute toxicity (LD_{50}) of the AS- SK2 antibiotic substance was assayed in six groups of six albino mice (18 – 25 body weight) per group, each respectively dosed intraperitoneally with graded concentrations of the antibiotics substance (0.48-7.60 mg/mlP.E) following the method of O'Callaghan (1983), with modification (Ekong *et al.,* 2004). All the mice were allowed access to food and water *ad libitum* and were observed for clinical signs of toxicity for 24 h. The control animals were dosed only with distilled water. Thereafter the LD_{50} of the AS-SK2 antibiotic substance was determined from formula of Lorke (1985), expressed as the square –root of the product of minimum dose showing 0% mortality (a) and maximum dose showing 100% mortality (b): ($LD_{50}= \sqrt{ab}$) (Lorke, 1985). The chemotherapeutic index, (C.I) was calculated as the ratio of the maximum tolerated toxic dose for mice and the minimum curative toxic dose for the test organisms (Ekong *et al.,* 2004).

***In-vivo* Mouse Protection Test against Establishment of Infections**

The ability of the AS-SK2 antibiotic substance to protect experimental animals separately dosed with the standardized broth culture of the test organisms was tested with albino mice (18-25 g body weight), following the Mouse Protection Test (MPT) of O'Çallaghan(1983), with modifications (Ekong *et al.,* 2004). The Mice (six per pathogen) were challenged intraperitoneally with 0.5 ml of 18-24 h culture of the pathogens, followed by the administration of the AS-SK2 antibiotic substance at the MIC dose, for an interval of one and five hours respectively. The animals were allowed access to food and water a*d libitum* for five days, during which they were monitored for clinical signs and morbidity.

RESULTS AND DISCUSSION

The antibacterial activity spectrum of the AS-SK2 antibiotic substance indicated a broad spectrum of activity against the staphylococci, especially the β-lactamase positive MRSA) and the other known β-lactamase producing Gram-positive and Gram-negative bacteria, with significantly ($p<0.05$) high potencies, compared with the controls (Table 1). This result could be of high clinical significance, since these test organisms are the etiologic agents of many mild, opportunistic, fatal, superficial and systemic infections (Fuerst, 1978; Konemann *et al.,* 1994; Ekong *et al.,* 2013). The broad spectrum and

highly potent activity of the AS-SK2 antibiotic substance against the test organisms is laudable in view of the increasing emergence of resistant bacterial strains to many antibiotics. The elaboration of bacterial enzymes, particularly β-lactamases, mostly with extended spectrum of activity, which often inactivate many antibiotics classes, as the most frequent resistant mechanism has been widely reported in both Gram-positive and Gram-negative bacteria (Davies, 1979; Neu, 1984; Werner 1985; Sanders and Watakunakorn, 1986; Spencer *et al.,* 1987; Sirot *et al.,* 1988; Phillipon *et al.,*1989; Main *et al.,* 1999, Livermore, 2003; Orbitsch *et al.,* 2004; Patterson *et al.,* 2004). Thus, the activity of the antibiotic substance against the staphylococci, especially the MRSA as well as the other test organisms, which are known β-lactamase producers, may possibly suggest either the exclusion of the antibiotic substance as a β-lactam; or regarded as a potentβ-lactamase inhibitor. In addition, the activity of the antibiotic substance against the biochemically recalcitrant MRSA, a well-known β-lactamase producer, may lend further support to this assertion of its exclusion as a β-lactam; or possibly as a β-lactamase inhibitor. Furthermore, the relatively excellent activity of the antibiotic substance against the Gram-negative bacterial species is laudable in view of their intrinsic resistance to antimicrobial agents, due to the possession of the recalcitrant and biochemically inert outer-membrane ((OM).The role(s) of OM as an intrinsic and biochemical resistance mechanisms, serving as a diffusion barrier preventing the passage of hydrophobic and large molecular weight hydrophilic substances, including antibiotics into the intracellular compartments of Gram-negative bacteria has been widely reported (Lieve, 1974; Nikaido, 1976; Nikaido *et al.,* 1983; Vaara and Vaara, 1983; Nikaido and Normark, 1987; Nikaido 1989; Gutmann *et al.,* 1985; Davies, 1979). The MIC, which confirmed the potency of the antibiotic substance, indicated a bacteriostatic activity which varied amongst the test organisms. It was the least (0.006 mg/ml PE) for SS, MRSA and SP (Gram-positive) and ST, SD and VC (Gram-negative) bacteria. Conversely, it was high 0.24 mg/ml PE and 0.48 mg/ml PE for EC and PA respectively compared with the controls (Table 2). This result showed that the Gram- positive bacteria tested recorded relatively lower MIC values than the Gram-negative bacteria to the antibiotic substance. This variation could be attributed to the intrinsic and biochemical resistance mechanisms in Gram-negative bacteria, due to the presence of the impervious OM, as the possibility of β-lactamase activity has been inhibited by the active inhibitor as already discussed. Evidently, the activity of the OM has been reported to concertedly increase antibiotics target active index (TAI) resulting in elevated MIC (Nikaido,1976; Nikaido and Normark, 1987; Nikaido, 1989; Ekong *et al.,* 2013). However, in this study, the MICs of the bioactive agent against the Gram-negative organisms tested were comparable to those of the Gram-positive organisms, but lower than that of the controls. This indicated high potency through the efficient penetration of the OM to the intracellular compartment. Contrarily, to the sensitivity of the Gram-negative bacteria tested to the AS-SK2 antibiotic substance, ST and SD were highly susceptible to the agent. This is laudable in view of the reported cross-resistance of these organisms to many antibiotics due to pleitropic mutations in the OM, resulting in porins-exclusion and reduced transport into the cell, leading to elevated MICs, due to increase in TAI (Nikaido, 1976; Nikaido and Normark, 1987; Nikaido, 1989; Ekong *et al.,* 2013). Thus, the high potency of the antibiotic substance against the test organisms could be explained on the likelihood of the effect of the bioactive agent on the OM, rendering it undulating thereby promoting free and efficient transportation of the antibiotic across the OM into the intracellular compartment. This action likely resulted in higher intracellular concentrations of the bioactive agent, hence the lower MICs recorded for the Gram-negative organisms. The lowest MIC values by the Gram-positive bacteria, especially the staphylococci, MRSA and BS, which are known β-lactamase producers is clinically interesting and further add credence to the potency of the antibiotic substance. The observed activity against PA, EC and MRSA is deemed significant given their generally known resistance, particularly β-lactamases production, with extended spectrum of activity against several antibiotics commonly used in chemotherapy. For instance, *Staphylococcus* aureus (SA), a known β-lactamase producer and a virulent pathogen that is responsible for a wide range of infections has developed resistance to most classes of antibiotics (Enright, 2003; Ekong *et al.,* 2013). Also , the occurrence of β-lactamase-positive *Staphylococcus aureus* (MRSA) in hospitals has risen from less than 3% in the early 1980's to as much as 40% now (Sahin and Ugur, 2003; Alanis, 2005; Enright, 2003; Ekong *et al.,* 2013). In addition, MRSA has been reported to have a prevalence of 10 – 50% in the USA hospitals, and to enjoy a comparable status in other countries (Brown an Reynolds; 1980; Mgbor *et al.,* 2002; Ekong *et al.,*2013). Also, it has been reported that the coagulase-negative staphylococci: SE and SS are increasingly becoming important in nosocomial infections and that they may cause serious infections (Kloos and Bannermann, 1994; Ekong *et al.,* 2013).These resistant staphylococci exhibit cross-resistance to all β-lactams as well as

other antibiotic classes such as erythromycin, tetracycline, chloramphenicol and gentamicin, etc. (Brown and Reynold, 1980; Reynold and Brown, 1985; Mgbor *et. al.*, 2002). Thus, the potency of the antibiotic substance against the MRSA, may possibly suggest the agent to be a very strong β-lactam or β-lactamase inhibitor. To this end, therapeutic options for infections by these resistant staphylococcal strains have been limited to vancomycin-based regiments. However, vancomycin therapy of staphylococcal infections has been associated with a slow and inadequate response in many cases, which presently the paradigm has changed, as vancomycin-resistant *Staphylococcus aureus* (VRSA) confronts clinicians worldwide (Levine *et al.,* 1991; Hiramatsu *et al.,* 1997; Hiramatsu, 1998; Bozdogan *et al.,* 2003; Chang *et al.,* 2003; Anonymous, 2004; Ekong *et al.,* 2013). Thus, the laudable activity of the AS- SK2 antibiotic substance against the staphylococci and MPSA, may be a promissory development in chemotherapy for the containment of these problematic pathogens. The AS-SK2 antibiotic substances in an *in vivo* test demonstrated to be relatively non-toxic, with an LD_{50} of 3.80 mg/ml P.E/kg, and a therapeutic index of 7.92 (Table 3). Of cardinal importance in the assessment of a new antibiotic substance for chemotherapy is its chemotherapeutic index(CI). This is because, if the chemotherapeutic index value is large, the antibiotic should be considered relatively safe; if it is small and close to unity, it may be safe under careful control dose; and if it one or less than one, the antibiotic should not be used clinically as chemotherapeutic agent (Rosenberg and Cohen 1983). Thus, with a chemotherapeutic index of 7.92, the antibiotic substance is considered relatively non-toxic, and may hold some promises on formulation as a potential chemotherapeutic agent. Most of the clinical toxicity symptoms observed such as toxic-shocks (nervousness) occurring immediately on administrations and characterized at the onset by rapid-breathing rate; high irritability followed later with extended-gait and drowsiness, may be attributed to hypersensitivity reactions apparently due to the presence of impurities, mainly fungal spores and hyphal elements in the crude antibiotic substance. This assertion is in line with the similar reported hypersensitivity reaction for other fungal and spore-forming bacterial antibiotic substance (Germaud and Touchaise, 1995; Mori *et.al.*, 1998; Kurup and Banner-Jee, 2000; Ekong *et.al.,* 2013). The rapid breathing rate and high irritability may be due to the anaphylactic response of immunoglobulin – E (IgE) antibody embedded in the mast-cells to the crude antibiotic solutions as similarly, reported for other fungal antibiotics and allergens (Duthie and Denning, 1995; Kurup and Bannerjee, 2000). This problem of impurities can be eliminated through the purification of the crude antibiotic substance. The ability or otherwise of the antibiotic-substance to offer protection to the experimental animals challenge intraperitoneally in the *in vivo* model of infection showed 100% protection of animals challenged with the staphylococci, SP and ST; 83.3% for those challenged with BS, SD and VC; 66.7% for those challenged with KP and PM; 33.33% for those challenged with EC; while all the animals challenged with PA, enjoyed no protection, and died after three days during the study period (Table 4). This result is laudable in that, the *in vivo* protection test in mice measured and confirmed the potency of the antibiotic substance in preventing the successful establishment of infections by the pathogens. The mouse protection test (MPT) has been reported to examine at the same time the *in-vivo* effects of serum-binding, metabolic/enzymatic conversion as well as the pharmacokinetics distribution of the antibiotics (O'Callaghan, 1983). Thus the result indicated that the antibiotic potency is not only limited to *in-vitro* situations, but is also applicable to *in vivo* situations. Moreover, pharmacokinetically, the MPT result in the study is significant as the laudable protection offered by the antibiotic may be indicative or confirmation of the absence of serum-binding, metabolic conversion and efficient systemic distributions of the antibiotic substance (Ekong *et al.,* 2004). Furthermore, the MPT indicated that the intraperitoneal injection could be a good route for the administration of the agent-in clinical trials, thereby circumventing the risk of metabolic conversion by intestinal enzymes during oral administration.

CONCLUSION

This study has shown that the AS-SK2 antibiotic substance elicited a broad-spectrum, bacteriostatic antibacterial activity with high potency and stability to the β-lactamase producing styaphylococci, especially the MRSA, as well as the other Gram-positive and Gram-negative bacteria. The broad-spectrum bacteriostatic β-lactam or β-lactamase inhibitor, was relatively non-toxic with a large therapeutic index, offering protection against the establishment of infections by the Gram-positive and Gram-negative β-lactamase producing pathogenic bacteria tested.

Table 1: Antibacterial activity spectrum and relative potency of Aspergillus SK2 Antibiotic substances (AS-Sk2).

Test organisms	Antibacterial activity/inhibition zone diameter, IZD (mm)			Relative Potency (%)	
	AS-SK2	PEN.G	CHL	PEN G	CHL
S.aureus (SA)	24.0	14.0	23.0	1.41	1.04
S. epidermidis (SE)	23.0	18.0	28.0	1.28	0.82
S. saprophyticus (SS)	25.0	16.0	25.0	1.56	1.00
Methicillin-resistant Staph. aureus`(MRSA)	26.0	19.0	23.0	1.36	1.13
B. subtilis (BS)	24.0	18.0	21.0	1.33	1.42
S.pyogenes (SP)	23.0	20.0	23.0	1.15	1.00
E. coli (EC)	14.0	12.0	21.0	1.16	0.67
S.typhi (ST)	26.0	18.0	28.0	1.44	0.98
P. aeruginosa (PA)	13.0	12.0	10.0	1.08	1.30
S. dysentariae SD)	26.0	20.0	23.0	1.30	1.13
V. cholerae (VC)	25.0	21.0	22.0	19	1.14
K. pneumoniae KP)	23.0	12.0	23.0	1.92	1.00
P. mirabilis (PM)	22.0	14.0	20.0	1.57	1.10

Table 2: MIC and mode of activity of AS – SK2 antibiotic substance

Test organisms	MIC of agents			MIC/MBC INDEX		
	AS – SK2 (mg/ml P.E)	Pen.G (mg/ml)	CHL (mg/ml)	AS-SK2	Pen. G	CHL
SA	0.12	0.13	12.50	+	-	+
SE	0.12	0.13	12.50	+	-	+
SS	0.06	0.13	12.50	+	-	+
MRSA	0.06	0.13	12.50	+	-	+
BS	0.12	0.13	12.50	+	-	+
SP	0.06	0.07	12.50	+	-	+
EC	0.24	0.52	25.0	+	-	+
ST	0.06	0.13	12.50	+	-	+
PA	0.48	0.52	25.0	+	-	+
SD	0.06	0.26	12.50	+	-	+
VC	0.06	0.26	12.50	+	-	
KP	0.12	0.26	25.0	+	-	+
PM	0.12	0.26	25.0	+	-	+

+ = presence of growth (-static activity); - = Absence of growth (cidal activity)

Table 3; Acute-toxicity (LD_{50}) of AS – SK2 Antibiotic substance

Quantity of AS- SK 2 administered (ml/kg body weight)	Relative dose of AS-SK2 administered (mg/ml P.E/kg)	No. of animals used	No. Of deaths within 24	% death recorded within 24h	LD_{50} (mg/ml P.E /Kg) body weight	C.I
0.5	0.48	6	-	0.00		
1.0	0.95	6	-	0.00		
2.0	1.90	6	-	0.00	3.80	7.92
4.0	3.80	6	2	33.33		
6.0	5.70	6	3	50.00	-	-
8.0	7.60	6	6	100.00		

= No death recorded; C. I. -= chemotherapeutic index

Table 4: Model Infection and Protection Test by AS – SK2 Antibiotic substance

Test Organisms	No. of animals used	No. of deaths within five day					Total No.of death per group	Total of No. of survivors per group	% death per group	% protection per group
		1	2	3	4	5				
SA	6	-	-	-	-	-	0	6	0.00	100.00
SE	6	-	-	-	-	-	0	6	0.00	100.00
SS	6	-	-	-	-	-	0	6	0.00	100.00
MRSA	6	-	-	-	-	-	0	6	0.00	100.00
BS	6	-	1	-	-	-	1	5	16.67	83.33
SP	6	-	-	-	-	-	0	6	0.00	100.00
EC	6	-	2	1	1	-	4	2	66.67	33.33
ST	6	-	-	-	-	-	0	6	0.00	100.00
PA	6	1	3	2	-	-	6	0	100.00	0.00
SD	6	-	1	-	-	-	1	5	16.67	83.33
VC	6	-	1	-	-	-	1	5	16.67	83.33
KP	6	-	1	1	-	-	2	4	33.33	66.67
PM	6	-	1	1	-	-	2	4	33.33	66.67

= no death recorded.

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