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Synthesis, Characterization and Antimicrobial Studies of Metal Complexes Derived from Gentamicin Sulfate

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The emergence of antimicrobial resistance has reduced the potential of gentamicin, which is a broad-spectrum aminoglycoside antibiotic. Complexation of gentamicin with metal ions is expected to facilitate the antibiotic discovery process and would overcome the antimicrobial resistance. Five metal complexes, Cr(III), Co(II), Ni(II), Cu(II) and Zn(II) complexes from gentamicin sulfate were successfully synthesized and characterized using decomposition point, elemental analyses, IR and UV–Vis spectroscopy. The results showed that all complexes have the general formula of $[ML_xL_y.aH_2SO_4.bH_2O]$, where M = metal ions (Cr, Co, Ni, Cu or Zn) and $L_x = L_y$ = gentamicin ligand of either L_1 = gentamicin C1 ($C_{21}H_{43}N_5O_7$) or L_2 = gentamicin C2 ($C_{20}H_{41}N_5O_7$) or L_3 = gentamicin C1a ($C_{19}H_{39}N_5O_7$). Characterization showed the presence of sulfuric acid molecules and coordinated water molecules in the metal complexes. Qualitative and quantitative antimicrobial assays were carried out to evaluate the biological activities of the parent compound and its metal complexes. It was found that all the complexes showed antimicrobial activity. The copper complex showed an increase of activity towards all the assayed microbial, while the chromium complex showed an enhanced activity with selectivity towards *S. pyogenes* and *K. pneumonia.*

Key words: Gentamicin; metal complexes; synthesis; antimicrobial activity

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Antimicrobial resistance (AMR) was declared by the World Health Organization (WHO) as one of the top ten global public health threats that required immediate action to achieve the Sustainable Development Goals (SDGs) [1]. This is because existing antibiotics are no longer effective with AMR, causing prolong illnesses that eventually will lead to disabilities and death. Thus, there is an immediate need to discover new antibiotics. The discovery of antibiotics can exhaust 20 years for just one effective antibiotics with the total antibiotic pipeline and stages of development [2].

Previous study showed that metal complexes from sulfonamides exhibited higher antibacterial activities compared to the parent sulfonamides [3]. Metal complexes of Schiff bases derived from sulfonamides also showed significant bioactivity when compared with the parent molecules [4]. It is well known that some metal ions are found to exhibit toxicity towards human health. However, our bodies still require traces of metal ions to maintain normal biological functions in the body [5]. We would like to exploit this and facilitate the discovery process by synthesizing metal complexes from existing antibiotics that exhibit AMR, thus, transforming them into potential new antibiotics.

Metals are well known to improve drug potency because they play roles as good chelating agents, stabilizes the compound, and can interact with biomolecules such as DNA, RNA, proteins, receptors, and lipids [6]. Metal ions act as cofactors for the compounds to reach their full activity in making the process of treatment more efficient. This is due to increase of lipophilicity in antibiotics which enhances the bacterial cell membrane penetration and blocking of metal-binding sites on enzymes [7].

Gentamicin is a well-known aminoglycoside antibiotic that exhibits bactericidal activity against aerobic gram-negative bacteria [8]. However, the emergence of gentamicin-resistant bacteria has caused a negative impact. The action mechanism of gentamicin towards aminoglycoside modifying enzymes is associated with the core structure of gentamicin, which consisted of various amino and hydroxyl functional group substitutions [8]. The presence of these functional groups has been reported to cause gentamicin to be able to coordinate metal ions easily [9]. Antimicrobial screening of the metal complexes derived from gentamicin have not been studied. Previous study only focused on the antibiotic susceptibility study of metal-gentamicin complexes against *Staphylococcus aureus* biofilms [10].

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Deduction from previous research on similar group of antibiotics inferred that the formation of metal complex can prevent mechanisms related to ototoxicity of gentamicin to occur.

In this study, transition metal complexes of gentamicin were synthesized by using chromium, cobalt, nickel, copper and zinc salts. Coordination modes of the complexes were investigated through various spectroscopic techniques and physical studies. The resultant antimicrobial activities of the coordination complexes were determined using qualitative and quantitative antimicrobial assays.

MATERIALS AND METHODS

All chemicals and solvents were of analytical grad and were used as received.

1. General Synthesis of Metal Complexes

The synthesis method used was modified from Mishra and Sharma [9]. Gentamicin sulfate (1.488 g, 1 mmol) dissolved in methanol (25 ml) was added to a solution of metal salt (1 mmol) dissolved in the same solvent (25 ml). The solution was stirred for 6 hours at room temperature until a precipitate was obtained. The excess solvent was filtered, and the precipitate washed thoroughly with excess methanol and dried over silica gel in a desiccator. The metal salts used were chromium(III) chloride hexahydrate (0.266 g), cobalt(II) acetate tetrahydrate (0.249 g), nickel(II) acetate tetrahydrate (0.249 g), copper(II) acetate monohydrate (0.200 g,) and zinc(II) acetate dihydrate (0.220 g).

2. Physical Measurements

Melting points were determined in an open capillary glass tube using an Electrothermal digital IA9000 Series melting point apparatus from room temperature until 400 °C. Carbon, hydrogen, nitrogen and sulfur analyses were carried out using a Perkin Elmer 2400 Series II CHNS Elemental Analyzer. The IR spectra were recorded in the range of 400-4000 cm^{-1} as KBr pellets on a Perkin Elmer Frontier FTIR spectrophotometer. The UV-Vis spectral analyses were recorded on a Perkin Elmer Lambda 35 UV-Vis spectrometer in a wavelength range of 200-800 nm using quartz cuvette. Metal determinations were carried out using a Perkin Elmer Analyst Atomic Absorption Spectrometer for chromium, nickel, copper and zinc complexes. Cobalt complex was analysed using ELAN ICP Mass Spectrometry to determine the cobalt content.

3. Target Microorganisms

Eight pathogens were used to test the biological potential of the ligands and metal complexes. They were *Staphylococcus aureus subsp. aureus* Rosenbach (ATCC 25923); *Klebsiella pneumoniae subsp. pneumoniae* (Schroeter) Trevisan (ATCC 700603), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Streptococcus pyogenes* Rosenbach (ATCC 19615) and *Candida albicans* (IMR C 523 11/A). The sources of microbes and culture maintenance were as previously described [10, 11] except for *Enterococcus faecalis* which was maintained on blood agar and overnight incubation at 37 ℃.

4. Qualitative Antimicrobial Assay

Antimicrobial activity of each compound was qualitatively determined by a modified disc diffusion method [11]. A lawn of microorganisms was prepared by pipetting and evenly spreading inoculum (10^{-4} cm^3, adjusted turbidometrically to $10^5 – 10^6$ cfu cm^3 (cfu: colony forming units) on to agar set in Petri dishes, using Mueller-Hinton agar (MHA). Sterile paper discs of 8 mm in diameter were impregnated with stock solution of gentamicin sulfate and its metal complexes in distilled water (100 mg cm^{-3}) to achieve a final concentration of 0.6 mg per disc and dried under sterile conditions. The dried discs were then placed aseptically and distinctively on the previously inoculated agar surface. The plates were inverted and incubated for 24 h at 30 °C for bacteria and 37 °C for fungi. Antimicrobial activity is indicated by the presence of clear inhibition zones around the discs. Commercially available streptomycin was used as antibacterial control and nystatin as antifungal control.

5. Quantitative Antimicrobial Assay

Positive antimicrobial compounds with inhibition zone ≥ 15 mm in disc diffusion assay were subjected to the quantitative measurement of microbiostatic (inhibitory) activity as described by Hufford and Clark [12] using a 96-well microtiter plate cell viability and the minimum inhibitory concentration (MIC) values of bacteria and fungi were determined by observing the turbidity and the absorbance reading using a VICTOR3 V 1420 Multilabel reader at 490 nm of the suspension. The lowest concentration, which completely inhibited visible microbial growth, was recorded as the minimum inhibitory concentration (MIC, µg cm^{-3}).

6. Determination of Minimum Bactericidal Concentration (MBC)/ Minimum Fungicidal Concentration (MFC)

The method used was modified from Siddiki *et al.* [13]. The MIC value obtained from the quantitative antimicrobial assay and the two concentrations above it for each of the compounds were cultured with 5 x 10^5 CFU cm^3 inoculum size in a sterile tube. The minimum bactericidal concentration (MBC) values and minimum fungicidal concentration (MFC) values was determined when there is no bacterial or fungal growth after five days incubation at 37 °C and 30 °C respectively.

RESULTS AND DISCUSSION

The physical and analytical data for gentamicin sulfate and its metal complexes are tabulated in Table 1. The elemental and metal analyses agreed well with the proposed structures. Gentamicin sulfate is comprised of three ligands, gentamicin C1, C1a and C2. All the synthesized complexes were obtained as solids, and were later found to be deliquescence because the solids kept without drying agent were found to be absorbing moisture until the solids dissolve in water. Our solubility testing showed that the complexes were very water soluble. They were neither dissolved in polar (methanol, ethanol, acetonitrile, DMSO, THF, DMF, pyridine) nor nonpolar organic solvents (hexane, chloroform, diethyl ether). This observation is different from gentamicin sulfate, which was quite soluble in polar solvents. All the complexes decomposed in the range of 243.4-247.3 ℃ (Table 1), suggesting that the complexes became unstable without the coordinated water molecule in the crystal lattice. The decomposition points of the complexes are higher than gentamicin sulfate, which melts between 218 and 237 °C [14] due to the presence of covalent dative bonds between the ligand and metal ion.

Important absorption bands in the IR spectra of these compounds are shown in Table 2. The FTIR spectra of gentamicin sulfate and metal complexes exhibited strong broad bands ranging from 3600 to 2600 cm^{-1} due to the presence of coordinated water molecules, the hydroxyl group, and the primary and secondary amine stretching vibrations. All the complexes showed a shift of the C-O stretching bands to a lower wavenumber compared to the IR spectrum of gentamicin sulfate, indicating the coordination of metal ions through deprotonated oxygen from the hydroxyl group. Gentamicin sulfate exhibited a band at 1618 cm^{-1} corresponding to the N-H bending. This band was shifted to a higher wavenumber for all the metal complexes, showing the participation of the amine group coordinating to the metal ions to form metal complexes [15].

Table 1. Physical and analytical data of the metal complexes

Compound	Color	Decomposition point (°C)	Found (calculated), %				Metal (%)
			C	H	N	S	
$CrL_1L_2.5H_2SO_4.18H_2O$	Dark green	247.1	27.11 (27.24)	7.85 (7.25)	7.86 (7.75)	9.85 (8.87)	2.41 (2.85)
$CoL_1L_3.5H_2SO_4.20H_2O$	Purple	247.3	26.75 (26.16)	7.39 (7.24)	7.60 (7.63)	9.46 (8.73)	3.64 (3.21)
$NiL_1L_2.6H_2SO_4.15H_2O$	Light green	245.2	27.00 (26.50)	6.15 (6.83)	7.83 (7.54)	9.27 (10.35)	2.45 (3.16)
$CuL_1L_3.5H_2SO_4.15H_2O$	Blue	243.7	27.50 (27.40)	7.87 (7.01)	7.93 (7.99)	10.05 (9.15)	4.26 (3.73)
$ZnL_1L_2.6H_2SO_4.12H_2O$	White	243.4	27.68 (27.19)	7.89 (6.68)	7.90 (7.73)	10.14 (10.62)	4.15 (3.61)

[a] L_1 = Gentamicin C1 ($C_{21}H_{43}N_5O_7$); L_2 = Gentamicin C2 ($C_{20}H_{41}N_5O_7$); L_3 = Gentamicin C1a ($C_{19}H_{39}N_5O_7$)

Table 2. Selected IR bands of gentamicin sulfate and its complexes

Compound	IR wavenumbers (cm^{-1})						λ_{max} (log ε)*
	ν(O-H)	δ(N-H)	ν(C-O)	ρ(H_2O)	ν(S=O)	δ(SO_2)	
Gentamicin sulfate	3414	1618	1124	-	1047	615	213 (1.600) 248 (1.431) 294 (1.275)
$CrL_1L_2.5H_2SO_4.18H_2O$	3400	1627	1116	746	1048	615	199 (3.427)
$CoL_1L_3.5H_2SO_4.20H_2O$	3414	1627	1116	748	1055	615	192 (3.139) 260 (2.427)
$NiL_1L_2.6H_2SO_4.15H_2O$	3400	1629	1116	750	1047	615	203 (2.644) 259 (1.773)
$CuL_1L_3.5H_2SO_4.15H_2O$	3400	1627	1116	755	1045	615	192 (3.346) 248 (2.934)
$ZnL_1L_2.6H_2SO_4.12H_2O$	3400	1627	1116	750	1051	615	200 (2.621)

*λ max (nm) and log ε (L mol-1 cm-1) are given in parentheses

The presence of coordinated water molecule in the metal complexes was evidenced with the bands in the region of 746 – 755 cm^{-1} corresponding to the rocking vibration of the coordinated water molecules. A strong intensity of the S=O stretching bands in the region of 1045 – 1051 cm^{-1} and the medium-to-weak bands of SO_2 bending at 615 cm^{-1} were attributed to the presence of sulfuric acid molecules in the metal complexes, which was identical to gentamicin sulfate.

From UV-Vis spectroscopy analyses, the band at approximately 294 nm, assigned as $\pi \rightarrow \pi^*$ transition of oxygen of the hydroxyl group from gentamicin had disappeared in all the complexes. Similar result was obtained showing the presence of this band in an uncoordinated ligand [9]. This disappearance of bands showed the involvement of the coordination through the hydroxyl group. This is further confirmed by the observation of bands in the region of 248-260 nm, which were assigned as $O^- \rightarrow Co(II)$, $O^- \rightarrow Ni(II)$ and $O^- \rightarrow Cu(II)$ transitions for the cobalt, nickel and copper complexes, respectively. The results indicate the binding of a deprotonated oxygen ligating group. The assignment of these bands was carried out on the basis of a previous study on copper(II) chelation by gentamicin [16]. The bands were observed in the region at 190 – 213 nm for gentamicin sulfate and all the complexes exhibited $n \rightarrow \sigma^*$ *transition of the connecting oxygen in the structure of purpurosamine, 2-deoxystreptamine and garosamine ring of the gentamicin [9]. This revealed the non-involvement of the oxygen atom of the 6-membered ring structure.* The shoulder band observed at 248 nm in ligands was assigned as the $n \rightarrow \pi^*$ transition across the cyanide (C-N) bond near the amine group of the gentamicin. No absorption was recorded in the visible region for all the complexes.

The antimicrobial assay results exhibiting the inhibition zone and the minimum inhibition concentration (MIC) values and the minimum bactericidal values (MBC) are tabulated in Tables 3 and 4. Gentamicin and its metal complexes were found to be very active against all the bacterial assayed with inhibition zones higher than or equal to 15.0 mm. However, none of them was active against *candida albicans*. This is not surprising because generally, fungi are not susceptible to the traditional aminoglycosides except at high concentrations [17]. From this finding, it is evident that the metal complexes of gentamicin did not alter the spectrum of activity of gentamicin sulfate against the fungal species because the core structure of the gentamicin was still there upon formation of the metal complexes. Spectrum of activity of an antimicrobial agent depends on the identity of structural formula of the compound [18]. A similar fingerprint region of the IR spectra for all complexes supported this reason.

The chromium complex exhibited larger inhibition zone diameters against *S. pyogenes* and *K. pneumoniae* compared to gentamicin sulfate by 2.4, and 3.7 mm respectively. These significance differences in the inhibition zone indicated an enhancement of antimicrobial activity upon formation of the chromium complex. These findings attested to the fact that the observed enhancement of antimicrobial activity of chromium complexes against *Staphylococcus aureus* and *Escherichia coli* was due to the chromium ion as the central ion for the coordinated complexes [19].

Table 3. Qualitative antimicrobial assay result

Compounds	Inhibition zone diameter* (mm) ± standard deviation					
	Bacterial					Fungal
	Gram-positive			Gram-negative		
	ATCC 25923	ATCC 19615	ATCC 29212	ATCC 25922	ATCC 700603	IMR C 523 11/A
Gentamicin sulfate	27.0± 1.0	34.3± 1.2	18.7± 1.5	26.3±2.1	16.0± 1.0	0
$CrL_1L_2.5H_2SO_4.18H_2O$	26.7±0.6	36.7± 1.5	16.8± 1.4	24.0 ± 0	19.7± 0.6	0
$CoL_1L_3.5H_2SO_4.20H_2O$	28.0 ± 0	33.7± 0.6	19.3± 1.2	25.3±0.6	16.0± 1.0	0
$NiL_1L_2.6H_2SO_4.15H_2O$	26.0 ± 0	31.7± 0.6	18.0 ± 1.0	25.3±0.6	15.2± 0.3	0
$CuL_1L_3.5H_2SO_4.15H_2O$	27.7± 0.6	36 ± 0	20.0 ± 1.0	27.3±0.6	17.0± 1.0	0
$ZnL_1L_2.6H_2SO_4.12H_2O$	27.0± 1.0	33.2± 1.8	16.7± 0.6	26.0±1.7	15.5± 0.9	0
Streptomycin (standard)	20.5± 0.7	20.5± 0.7	12.5± 0.7	25.5±0.7	22.0 ± 0	ND
Nystatin (standard)	ND	ND	ND	ND	ND	20.0± 0
Distilled water (control)	0	0	0	0	0	0

*Diameter of 15 mm and above is considered active

ND denoted for not determined.

Staphylococcus aureus subsp. aureus Rosenbach (ATCC 25923); *Klebsiella pneumoniae subsp. pneumoniae* (Schroeter) Trevisan (ATCC 700603), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Streptococcus pyogenes* Rosenbach (ATCC 19615) and *Candida albicans* (IMR C 523 11/A).

Table 4. MIC and MBC values in µg/ml of all the compounds against tested organisms

Compounds	Bacteria*									
	ATCC 25923		ATCC 19615		ATCC 29212		ATCC 25922		ATCC 700603	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gentamicin sulfate	0.2	0.8	0.1	0.4	12.8	51.2	0.8	3.2	6.4	25.6
$CrL_1L_2.5H_2SO_4.18H_2O$	0.2	0.8	0.1	0.2	12.8	51.2	0.8	3.2	6.4	12.8
$CoL_1L_3.5H_2SO_4.20H_2O$	0.2	0.8	0.1	0.4	12.8	51.2	0.8	3.2	6.4	25.6
$NiL_1L_2.6H_2SO_4.15H_2O$	0.2	0.8	0.1	0.4	12.8	51.2	0.8	3.2	6.4	25.6
$CuL_1L_3.5H_2SO_4.15H_2O$	0.2	0.8	0.1	0.2	12.8	25.6	0.8	1.6	6.4	25.6
$ZnL_1L_2.6H_2SO_4.12H_2O$	0.2	0.8	0.1	0.4	12.8	51.2	0.8	3.2	6.4	25.6

MIC (µg/ml) = Minimum Inhibitory Concentration, i.e. the lowest concentration to completely inhibit microbial growth

MBC (µg/ml) = Minimum Bactericidal Concentration, i.e. the lowest concentration to completely inhibit microbial growth

**Staphylococcus aureus subsp. aureus* Rosenbach (ATCC 25923); *Klebsiella pneumoniae subsp. pneumoniae* (Schroeter) Trevisan (ATCC 700603), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Streptococcus pyogenes* Rosenbach (ATCC 19615) and *Candida albicans* (IMR C 523 11/A).

Copper complex exhibited the most significant enhancement in antimicrobial activity compared to gentamicin sulfate. This enhanced activity of the complex was in accordance with the findings on copper complex of cefixime antibiotic towards these gram-positive and gram-negative bacteria [7]. It was also found that the cobalt complex exhibited a slightly higher activity compared to gentamicin sulfate against *S. aureus* and *E. faecalis*. However, these differences were not significant because the MBC values measured were the same.

Both nickel and zinc complexes showed either comparable or lower inhibition zones against all the assayed bacteria compared to the parent compound. This finding is in contradiction with literature which showed that the zinc complex was found to be active against *E. coli, K. pneumoniae* and *S. aureus* [7], whereas nickel complex was found to be antimicrobial active against most gram-positive bacteria [20]. Our result suggested that the antimicrobial activity of complexes was reduced upon complexation of gentamicin sulfate with zinc and nickel ions.

Comparison of the MIC values of gentamicin sulfate and its metal complexes is shown in Table 4, indicating that the complexes exhibited the same trend of bacteriostatic activity as the parent compound with the same MIC values. The MIC values, arranged in the order of their effectiveness as a bacteriostatic agent against bacterial species are 0.1 µg/ml (*S.* pyogenes), 0.2 µg/ml (*S. aureus*), 0.8 µg/ml (*E. coli*), 6.4 µg/ml (*K. pneumoniae*) and 12.8 µg/ml (*E. faecalis*). However, the trend of the MBC values was slightly different. The chromium complex exhibited high bactericidal activity against *S.* pyogenes (0.2 µg/ml) and *K. pneumoniae* (12.8 µg/ml) compared to gentamicin sulfate while the copper complex was found to display high bactericidal activity against *S. pyogenes* (0.2 µg/ml), *E. faecalis* (25.6 µg/ml) and *E.coli* (1.6 µg/ml). The cobalt, nickel and zinc complexes were found to possess the same MBC values as the parent compound. Both gentamicin sulfate and its complexes were observed to have the same bactericidal activity against *S. aureus* (0.8 µg/ml).

It is concluded that the chromium complex showed better bactericidal activity against *S. pyogenes* and *K. pneumoniae*, and the copper complex showed bactericidal activity against *S. pyogenes, E. faecalis* and *E. coli*, when compared to gentamicin sulfate. The enhancement in antimicrobial activity of copper and chromium complexes can be related to the theory stated by Pillai and Latha [7]. Upon chelation, the polarity of the metal ion will be reduced to a greater extent due to overlapping of the ligand orbital and partial sharing of the positive charge of the metal ion with the donor groups. Further, it increases the delocalization of π-electrons over the whole chelate ring and thus enhances the lipophilicity of the complexes. The increased lipophilicity enhances the permeation of the complexes into liquid membranes thereby blocking the metal binding sites in the enzymes of the microorganisms. The complexes also disturb the respiration process of the cell which then block the synthesis of the proteins that restricts the growth of the organism. There are hypothesis postulating that Cu ions are harnessed directly by phagocytes. Within the reducing bacterial cytoplasm, Cu(II) ion can be converted to Cu(I) and its level is buffered by Cu(I)-specific, high-affinity sites. This excess of Cu(I) ion exerts a bacteriotoxic effect via an alternative redox-independent mechanism [21].

CONCLUSION

Chromium, cobalt, copper, nickel and zinc complexes were synthesized from gentamicin sulfate and successfully characterized using physical and spectroscopic methods. All complexes have a general formula of $[ML_xL_y.aH_2SO_4.bH_2O]$, where M = metal ions (Cr, Co, Ni, Cu or Zn) and $L_x = L_y$ = gentamicin ligand of either L_1 = gentamicin C1 ($C_{21}H_{43}N_5O_7$) or L_2 = gentamicin C2 ($C_{20}H_{41}N_5O_7$) or L_3 = gentamicin

C1a ($C_{19}H_{39}N_5O_7$). Spectroscopic analyses confirmed that the coordination of the metal ions occurred through the amino and hydroxyl groups of gentamicin. The coordination sphere showed the presence of sulfuric acid molecules and coordinated water molecules in the metal complexes. The chromium and copper complexes exhibited an increase of antimicrobial activity. The zinc and nickel complexes showed comparable or some decrease in antimicrobial activity when compared to gentamicin sulfate, whereas the cobalt complex showed an insignificant increase of activity. Thus, the chromium and copper complexes can function as potential antibacterial agents.

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